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SOCIETY OF PERINATAL OBSTETRICIANS



1992 12th ANNUAL MEETING

Scientific, Clinical, and Business Meeting

February 3-8, 1992
Orlando, Florida

28 OCT 1992
28

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12th Annual Meeting

of the

Society of Perinatal Obstetricians

February 3-8, 1992

Orlando, Florida

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In accordance with the wishes of the majority of the SPO membership, we ask that our members and guests refrain from smoking in the meeting rooms. This conforms to the spirit of our society and the written procedures of our parent organization.

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12th Annual Clinical, Scientific, & Business Meeting

of the

SOCIETY OF PERINATAL OBSTETRICIANS

February 3–8, 1992
Walt Disney World Hilton
Orlando, Florida

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Society of Perinatal Obstetricians

409 12th STREET, SW
WASHINGTON, DC 20024
(202) 863-2476

Dear Colleagues:

The 1992 SPO Meeting, like the 1991 SPO Meeting, had a record number of abstracts submitted. This year we had a total of 881 abstracts, which is 93 (11%) more than 1991. A total of 664 abstracts or 75% of those submitted were accepted compared to 86% last year. Of special interest was the fact that we had a large number of abstracts (69) submitted from outside the continental United States. The breakdown of the 881 abstracts submitted for the 1992 SPO Annual Meeting is summarized below:

Abstracts submitted	881
Oral presentations	44 (5%)
Poster presentations	491 (56%)
Published, not presented	129 (15%)
Rejected	217 (25%)*

* Includes those not published at the authors' request.

In accordance with guidelines established over the last two years, each abstract was judged by four qualified reviewers, selected from the regular SPO membership. In no case was a reviewer's evaluation used to judge his/her own abstract for the final scoring and in no case was there an institutional conflict. As has been our policy, the abstracts were judged without knowledge of the authors or institutions. My personal thanks to the reviewers for their carefully considered judgments and speedy responses (although a gentle nudge was sometimes required). I would also like to personally thank whoever invented the FAX machine.

Very special thanks and gratitude go to Lynne McDonnell, Administrative Assistant for Maternal-Fetal Medicine, for her skillful organization and hundreds of hours of work which went into preparing this syllabus. I would also like to give personal thanks to Laurie Daniels and Terry Daniels who assisted Lynne in this endeavor. Ms. Patricia Stahr of our SPO Headquarters in Washington, D.C. is deserving of special recognition for all of her hard work and wonderful advice in planning and preparing for this meeting. These four key individuals have made being the 1992 Program Chair a pleasure instead of a nightmare! Finally, I would like to give thanks to our President, Dr. Thomas Garite, and all of the members of the Board of Directors for their enthusiastic support and assistance in planning this meeting.

As a final note, it would appear that our "crisis" regarding future meeting sites has ended. In 1993 the SPO Meeting will be held at the San Francisco Hilton, San Francisco, CA and in 1995 it will be held at the Atlanta Hilton in Atlanta, GA. The 1994 meeting will remain in Las Vegas at the Las Vegas Hilton.

Sincerely,

Larry C. Gilstrap, III, M.D.
Program Chair, 1992 SPO Annual Meeting



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Local Arrangements Chair

The Program Chairman, on behalf of the Society, is most grateful to the following people who so conscientiously and promptly judged the abstracts for this meeting.

Iffath Abbasi-Hoskins, M.D.
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George D. Wendel, M.D.
John Williams, III, M.D.
James R. Woods, M.D.
Bruce A. Work, M.D.
Sze-ya Yeh, M.D.
Edward R. Yeomans, M.D.
Bruce K. Young, M.D.

Acknowledgements

The Society of Perinatal Obstetricians wishes to express thanks and deep appreciation to the following organizations for their generous support of the 1992 Annual Meeting(*):

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Vivigen
Wiley-Liss

(*) This list reflects known contributors as of press deadline.

Awards

n behalf of the Society of Perinatal Obstetricians, the 1992 Program Committee will announce the following awards as outstanding contributions to this meeting at the Annual Banquet on Thursday, February 6:

Society of Perinatal Obstetricians Award

$1000

Outstanding Research by a Fellow-in-Training

Sponsored by the Society of Perinatal Obstetricians
$1000

Best Genetics Research in the Field of Perinatal Medicine

Sponsored by Vivigen
$500

Best Doppler Research in the Field of Perinatal Medicine

Sponsored by Multigon Industries
$500

****

Awards for the Best Poster Presentations

Sponsored by the Society of Perinatal Obstetricians
$500

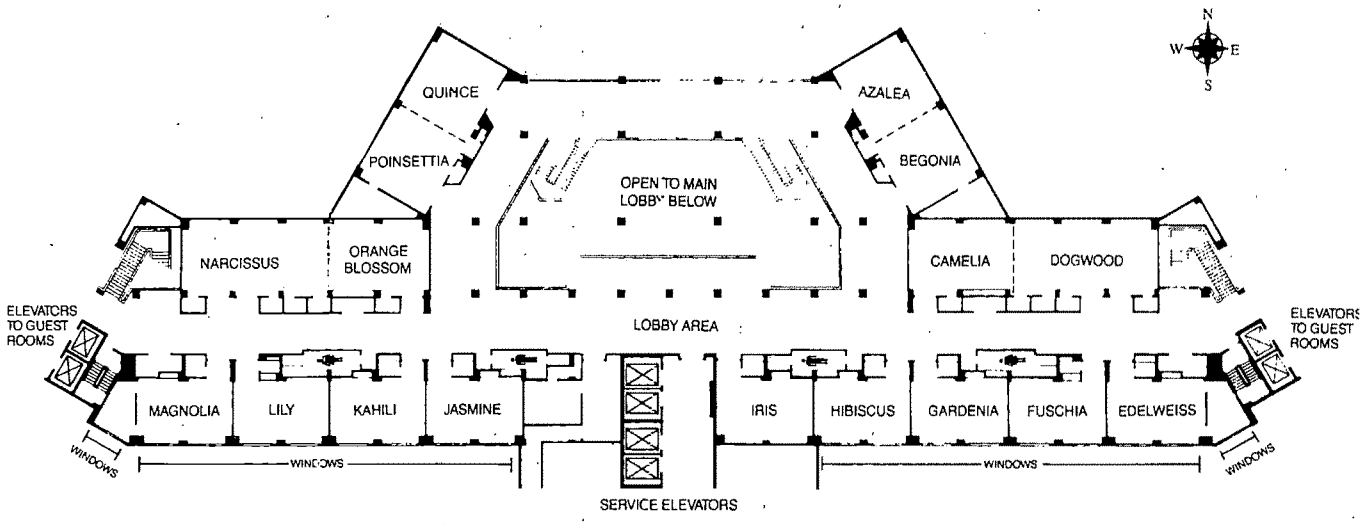
award will be given to the best poster presented at each of the five sessions. These awards will be announced during the closing ceremony on Saturday, February 8, 1992.

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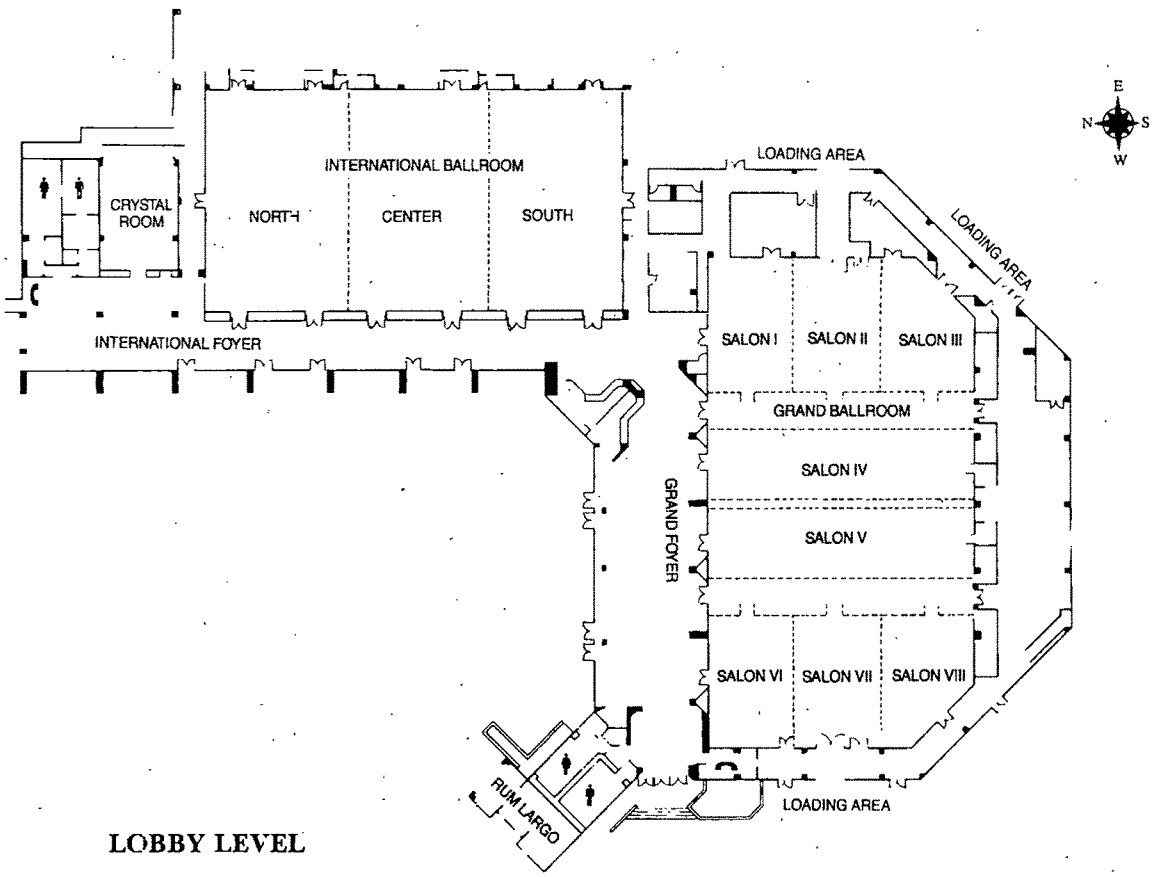
Humana Award for Excellence

iis award is for the physician who is extensively involved with the direct care of patients, and who also has the ive and energy to conduct clinical research resulting in a significant advance in diagnosis and treatment. The recipient, selected by the SPO, will be announced at the Thursday Banquet.

HILTON AT WALT DISNEY WORLD VILLAGE



MEZZANINE LEVEL



LOBBY LEVEL

SOCIETY OF PERINATAL OBSTETRICIANS

12th Annual Meeting—February 3-8, 1992
Walt Disney World Hilton—Orlando, Florida

Program

Monday, February 3, 1992

6:00 pm-8:00 pm	Registration	East/West Registration

Tuesday, February 4, 1992

7:00 am-7:00 pm	Registration	East/West Registration
7:00 am-8:00 am	Continental Breakfast	Grand Foyer + Pool Deck
7:00 am-7:00 pm	Speaker Ready Room	Lily
8:00 am-4:00 pm	**Postgraduate Course I** *"Medical Complications of Pregnancy"*	Grand Salons I-II-III
	Postgraduate Course II *"Genetics"*	Grand Salons IV-V
9:30 am-10:00 am	Coffee Break	Grand Foyer + Pool Deck
12:00 pm-1:00 pm	Lunch	Center/South Ballrooms
2:30 pm-3:00 pm	Coffee Break	Grand Foyer + Pool Deck
4:00 pm-7:00 pm	**Board of Directors Meeting I**	Poinsettia/Quince
7:00 pm-9:30 pm	**Board of Directors Dinner**	

Wednesday, February 5, 1992

7:00 am-6:00 pm	Registration	East/West Registration
7:00 am-8:00 am	Continental Breakfast	Grand Foyer + Pool Deck
7:00 am-7:00 pm	Speaker Ready Room	Lily
8:00 am-3:00 pm	**Postgraduate Course III** *"The Fetus as a Patient"*	Grand Salons I-II-III
	Postgraduate Course IV *"Fetal Echo/Doppler Velocimetry"*	Grand Salons IV-V
	Postgraduate Course V *"Viral Infections in Pregnancy"*	Grand Salons VI-VII-VIII
10:00 am-10:30 am	Coffee Break	Grand Foyer + Pool Deck
12:00 pm-1:00 pm	Lunch	International Ballroom
12:00 pm-3:00 pm	**Board of Directors Meeting II**	Jasmine
3:00 pm-5:00 pm	**Special Interest Group Meetings**	(see page xiv for locations)
5:00 pm-6:00 pm	**Program Directors Meeting**	Grand Salons VI-VIII
7:00 pm-10:00 pm	Cocktail Reception	International Ballroom

Thursday, February 6, 1992

7:00 am-5:00 pm	Registration	East/West Registration
7:00 am-7:45 am	Continental Breakfast	Grand Foyer + Pool Deck
7:00 am-7:00 pm	Speaker Ready Room	Lily
7:45 am-8:00 am	**Welcome and Announcements** Larry C. Gilstrap, III, M.D. Program Chairman, 1992 SPO **Welcome** Thomas J. Garite, M.D. President, SPO 1992	Grand Salons V-VIII
8:00 am-10:30 am	**Oral Session I** Moderator: Thomas J. Garite, M.D. President, SPO 1992	Grand Salons V-VIII
10:30 am-12:00 pm	**Poster Session I & Coffee**	Grand Salons I-IV
12:00 pm-12:30 pm	**Poster Session Discussion** Moderator: Baha Sibai, M.D.	Grand Salons I-IV
12:30 pm-2:00 pm	Lunch	International Ballroom
2:00 pm-4:00 pm	**Oral Session II** Moderator: Julian T. Parer, M.D., Ph.D. Program Chairman, SPO 1991	Grand Salons V-VIII
4:00 pm-6:00 pm	**Poster Session II & Coffee**	Grand Salons I-IV
5:30 pm-6:30 pm	**Annual SPO Business Meeting** (SPO Members Only)	Grand Salons V-VIII
7:00 pm-8:00 pm	Cocktail Reception	Grand/Int'l Foyers + Pool Decks
8:00 pm-10:30 pm	**Annual Banquet**	International Ballroom
10:30 pm-12:30 am	Dessert & Dancing	Grand Salons V-VIII

Friday, February 7, 1992

7:00 am-5:00 pm	Registration	East/West Registration
7:00 am-8:00 am	Continental Breakfast	Grand Foyer + Pool Deck
7:00 am-8:00 am	**Special Interest Group Breakfasts**	(see page xiv for locations)
7:00 am-7:00 pm	Speaker Ready Room	Lily
8:00 am-10:00 am	**Oral Session III** Moderator: Garland Anderson, M.D. President Elect, SPO	Grand Salons V-VIII
10:00 am-12:00 pm	**Poster Session III & Coffee**	Grand Salons I-IV
12:00 pm-12:30 pm	**Poster Session III Discussion**	Grand Salons I-IV
12:30 pm-2:00 pm	Lunch	International Ballroom
2:00 pm-4:00 pm	**Oral Session IV** Moderator: Sze-ya Yeh, M.D. Secretary/Treasurer, SPO	Grand Salons V-VIII
4:00 pm-6:00 pm	**Poster Session IV & Coffee**	Grand Salons I-IV

Saturday, February 8, 1992

7:00 am-12:00 pm	Registration	East/West Registration
7:00 am-8:00 am	Continental Breakfast	Grand Foyer + Pool Deck
7:00 am-1:00 pm	Speaker Ready Room	Lily
8:00 am-10:30 am	**Oral Session V** Moderator: Frank C. Miller, M.D. Past President, SPO	Grand Salons V-VIII
10:30 am-12:00 pm	**Poster Session V & Coffee**	Grand Salons I-IV
12:00 pm-12:30 pm	**Poster Session V Discussion** **Announcement of Poster Awards** Adjourn	Grand Salons I-IV

SPECIAL INTEREST GROUP MEETINGS

Wednesday, February 5, 1992
3:00–5:00 p.m.

1) ***NORTH AMERICAN SOCIETY FOR THE STUDY OF HYPERTENSION IN PREGNANCY** Coordinator: Baha Sibai	North Ballroom
2) **DIABETES MELLITUS IN PREGNANCY** Coordinator: Larry Cousins	Narcissus/Orange Blossom
3) **INFECTIOUS DISEASES IN PERINATAL MEDICINE** Coordinator: Bernard Gonik	Crystal
4) **GENETICS IN PERINATOLOGY** Coordinator: Karin Blakemore	Poinsettia/Quince
5) **ULTRASOUND IN PERINATAL MEDICINE** Coordinator: Isabelle Wilkins	Camellia/Dogwood
6) **COMMUNITY HOSPITAL BASED PERINATOLOGISTS** Coordinator: Federico Mariona *NOTE: Time will be extended to 6:00 p.m.*	Iris
7) **COMPUTER USAGE IN PERINATAL MEDICINE** Coordinator: Sidney Bottoms	Kahili
8) **CRITICAL CARE IN OBSTETRICS** (DISCONTINUED—COMBINED WITH HYPERTENSION GROUP)	
9) **PRETERM LABOR** Coordinator: Robert Creasy	Azalea/Begonia

---

Friday, February 7, 1992
7:00–8:00 a.m.
(Breakfast Meetings)

10) **WOMEN IN PERINATOLOGY** Coordinator: Sue Palmer	Camellia/Dogwood
11) **INTERNATIONAL PERINATAL OBSTETRICIANS** Coordinator: Bruce Work	Poinsettia/Quince

**NASSHP will have an additional meeting on Wednesday evening from 7:00-9:00 pm in Grand Salons VI-VII-VIII.*

SOCIETY OF PERINATAL OBSTETRICIANS

Orlando, Florida – February 3-8, 1992

Schedule of Oral Presentations

Thursday, February 6, 1992

8:00am-10:30am	Oral Session I: Prematurity; Labor; Neonatology Moderator: *Thomas J. Garite, M.D.* *President, SPO*	Grand Salons V-VIII

8:00-8:15am	1	ONCOFETAL FIBRONECTIN IN PATIENTS AT INCREASED RISK FOR PRETERM DELIVERY	*Michael P. Nageotte, K.A. Hollenbach, B.A. Vanderwahl, K.M. Hutch* Long Beach Memorial Womens Hospital, Long Beach, CA and University of California, Irvine
8:15-8:30am	2	NATURAL INTERLEUKIN-1 RECEPTOR ANTAGONIST BLOCKS INTERLEUKIN-1-INDUCED PROSTAGLANDIN PRODUCTION BY HUMAN INTRAUTERINE TISSUES: THE BASIS FOR A NOVEL APPROACH TO THE TREATMENT OF PRETERM LABOR IN THE SETTING OF INFECTION	*Roberto Romero, W. Sepulveda, M. Mazor, C. Dinarello, M. Mitchell* Yale University, New Haven, CT and University of Utah, Salt Lake City, UT
8:30-8:45am	3	KETEROLAC BLOCKS RITODRINE-STIMULATED PRODUCTION OF $PGF_{2\alpha}$ IN PREGNANT SHEEP	*Phillip N. Rauk, Steven A. Laifer* University of Pittsburgh, Magee Women's Hospital Pittsburgh, PA
8:45-9:00am	4	CESAREAN SECTION FOR FETAL INDICATIONS AT THE LIMITS OF FETAL VIABILITY (1986 TO 1991)	*Erol Amon, Sam Moyn* St. Louis University, St. Mary's Health Center St. Louis, MO
9:00-9:15am	5	NEONATAL INTRAVENTRICULAR HEMORRHAGE (IVH) FOLLOWING MATERNAL BETA-SYMPATHOMIMETIC TOCOLYSIS	*Lynn J. Groome, Robert L. Goldenberg, S.P. Cliver, R.O. Davis, R.L. Copper* University of Alabama Birmingham, AL
9:15-9:30am	6	THE CANADIAN MULTICENTRE RCT OF EARLY AMNIOTOMY	*William D. Fraser, S. Marcoux, J.M. Moutquin, A. Christen, B.A. Armson, J.P. Verreault, N. Okun, C. Nimrod, A.K. Joshi, H. Cohen, L. Bayer, T. Doran, P. Bernstein, J. Carroll, S. Bottoms, F. Galerneau* Laval University Quebec, Canada
9:30-9:45am	7	EXPRESSION OF PARATHYROID HORMONE-RELATED PEPTIDE (PTHrP) mRNA IN PLACENTAL MEMBRANES AND AMNIOTIC FLUID (AF)	*J.E. Ferguson II, J. Gorman, D.E. Bruns, M.R. Pandian, M.E.H. Bruns* University of Virginia, Charlottesville, VA and Nichols Institute, San Juan Capistrano, CA

9:45-10:00am	8	COMPARISON OF INDUCTION METHODS FOR PREMATURE RUPTURE OF MEMBRANES AT TERM	*J.F. McCaul, L.M. Williams, R.W. Martin, E.F. Magann, L. Gallagher, J.C. Morrison* University of Mississippi Jackson, MS
10:00-10:15am	9	DOES ANTENATAL MATERNAL BETAMETHASONE ADMINISTRATION REDUCE NEONATAL MORBIDITY FOLLOWING IMMEDIATE SURFACTANT THERAPY AT DELIVERY?	*Andrew W. Davey, David M. Sherer, Jacques S. Abramowicz, Christopher Cox, James W. Kendig* University of Rochester Rochester, NY
10:15-10:30am	10	UMBILICAL ARTERY CREATINE KINASE BRAIN BAND % PREDICTS MAJOR INTRAVENTRICULAR HEMORRHAGE	*R. Figueroa, L. Gonzalez, U. Verma, R. Carter, I. Argani, N. Tejani* New York Medical College Valahalla, NY

Thursday, February 6, 1992

2:00pm-4:00pm		**Oral Session II: Medical Complications of Pregnancy; Hypertensive Disease of Pregnancy** **Moderator:** ***Julian T. Parer, M.D., Ph.D.*** ***Program Chair, 1991 SPO***	**Grand Salons V-VIII**
2:00-2:15pm	11	THRESHOLD VALUES FOR GLUCOSE TOLERANCE TEST (GTT) IN PREGNANCY NEED TO BE MODIFIED	*Nina Boe, J. Dacus, B. Mercer, K. Schulz, B. Sibai* University of Tennessee Memphis, TN
2:15-2:30pm	12	NEONATAL OUTCOME IN PREGNANCIES COMPLICATED BY HYPERTHYROIDISM	*Lynnae Millar, D. Wing, P. Koonings, M. Montoro, J. Mestman* University of Southern California Los Angeles, CA
2:30-2:45pm	13	ANTIPHOSPHOLIPID SYNDROME. OUTCOME OF TREATED PREGNANCIES: AN UPDATE OF THE UTAH EXPERIENCE	*Robert M. Silver, D.W. Branch, D.J. Dudley, J.R. Scott* University of Utah Medical Center Salt Lake City, UT
2:45-3:00pm	14	ACETYLSALICYLIC ACID INHIBITS ANTICARDIOLIPIN ANTIBODY-INDUCED PLATELET-ACTIVATING FACTOR SYNTHESIS	*Richard K. Silver, P.D. O'Connell, M.S. Caplan* Northwestern University Medical School Evanston, IL
3:00-3:15pm	15	A RANDOMIZED PROSPECTIVE COMPARISON OF NIFEDIPINE AND BED REST VERSUS BED REST ALONE IN THE MANAGEMENT OF PREECLAMPSIA REMOTE FROM TERM	*Baha M. Sibai, J.R. Barton, S. Aki, C. Sarinoglu, B.M. Mercer* University of Tennessee Memphis, TN
3:15-3:30pm	16	MAGNESIUM SULFATE INJECTIONS BLOCK NMDA-INDUCED HIPPOCAMPAL SEIZURES	*David B. Cotton, R.F. Berman, S. Irtenkauf* Wayne State University/Hutzel Hospital Detroit, MI

3:30-3:45pm	17	MAGNESIUM PIDOLATE INFUSION REDUCES ANGIOTENSIN II PRESSOR RESPONSE IN PREGNANT WOMEN	*Andrea L. Tranquilli, M.L. Mariani, C.G. Garzetti, H. Valensie, C. Romanini* University of Ancona Ancona, Italy
3:45-4:00pm	18	A PROSPECTIVE STUDY OF BIOIMPEDANCE ANALYSIS IN NORMAL AND HYPERTENSIVE PREGNANCIES	*Thomas Murphy Goodwin, S. Estrada, K.A. Smith, L. Bernstein, R. Artal* University of Southern California Los Angeles, CA

Friday, February 7, 1992

8:00am-10:00am		**Oral Session III: Genetics & Teratology; Fetal Therapy; Placental Physiology** **Moderator:** ***Garland Anderson, M.D.*** ***President Elect, SPO***	**Grand Salons V-VIII**
8:00-8:15am	19	A PROSPECTIVE EVALUATION OF TRIPLE MARKER MATERNAL SERUM SCREENING FOR TRISOMY-21	*E.Y. Cheng, D.A. Luthy, D.E. Hickok, R. Lieppman, R.G. Resta, M. Williams, A. Zebelman, F. Luthardt* Swedish Hospital Medical Center Seattle, WA
8:15-8:30am	20	MID-TRIMESTER ECHOGENIC BOWEL AND CHROMOSOMAL ABNORMALITIES	*Angela Scioscia, D. Pretorius, N. Budorick, T. Cahill, F. Axelrod, G. Leopold* University of California, San Diego San Diego, CA
8:30-8:45am	21	PREGNANCY LOSS AFTER FIRST TRIMESTER ULTRASONOGRAPHIC DOCUMENTATION OF EMBRYONIC/FETAL CARDIAC ACTIVITY	*Jeffrey M. Barrett, Jennifer Brinson* Watson Clinic Lakeland, FL
8:45-9:00am	22	EVALUATION OF FETAL BLOOD CONTENT IN TRANSABDOMINAL AND TRANSCERVICAL CHORIONIC VILLUS SAMPLES	*Karin Blakemore, I. Baser, N. Callan, R.S. Shirey, T. Kickler, M. Blitzer* The Johns Hopkins University and University of Maryland Baltimore, MD
9:00-9:15am	23	DIAGNOSIS AND TREATMENT OF TWIN TO TWIN TRANSFUSION SYNDROME (TTTS)	*Carl Weiner, A. Ludomirski* University of Iowa Hospital, Iowa City, IA and Pennsylvania Hospital, Philadelphia, PA
9:15-9:30am	24	LYMPHOCYTE SUBSETS IN PRENATALLY OBTAINED FETAL BLOOD	*Stanley M. Berry, J. Kaplan, N.L. Fine, J.A. Bichalski, M.P. Dombrowski, N.B. Isada, M.I. Evans, D.B. Cotton* Wayne State/Hutzel Hospital and Children's Hospital of Michigan Detroit, MI

9:30-9:45am	25	PRODUCTION OF ENDOTHELIN-1 BY HUMAN TROPHOBLASTS	*Philip Samuels, J.D. Steinfeld, M. Rhoa, S. Murray, J. Amico, D.B. Cines, K.R. McCrae* University of Pennsylvania Philadelphia, PA and University of Pittsburgh Pittsburgh, PA
9:45-10:00am	26	THE EFFECTS OF LOW-DOSE ASPIRIN ON PROSTACYCLIN AND THROMBOXANE PRODUCTION BY THE PERFUSED HUMAN PLACENTA	*Robert L. Jacobson, Anthony Brewer, Tariq A. Siddiqi, Leslie Myatt* University of Cincinnati Cincinnati, OH

Friday, February 7, 1992

2:00pm-4:00pm	**Oral Session IV: Clinical/Operative Obstetrics; Ultrasound; Infectious Disease** **Moderator:** ***Sze-ya Yeh, M.D.*** ***Secretary-Treasurer, SPO***	**Grand Salons V-VIII**

2:00-2:15pm	27	A SIGNIFICANT REDUCTION IN CESAREAN DELIVERIES: EFFECT ON PERINATAL OUTCOME	*Luis Sanchez-Ramos, Mark T. Cullen, Carol Walker* University of Florida Jacksonville, FL
2:15-2:30pm	28	VAGINAL DELIVERY OF THE NON-VERTEX SECOND TWIN	*Alan Fishman, Debra Grubb, Bruce Kovacs* University of Southern California Los Angeles, CA
2:30-2:45pm	29	PRENATAL CARE: DIFFERENTIAL EFFECTS ON MATERNAL AND NEONATAL OUTCOMES	*J.W. Sparks, James A. McGregor, M.G. Leff, D.C. Lezotte, M. Orleans* University of Colorado Health Sciences Center Denver, CO
2:45-3:00pm	30	INTRAUTERINE GROWTH RETARDATION: A COMPARISON OF THE 3RD VERSUS 10TH PERCENTILE	*Susan L. Baker, John C. Hauth, Robert L. Goldenberg, S.P. Cliver, R.L. Copper* University of Alabama Birmingham, AL
3:00-3:15pm	31	EVALUATION OF DIFFERENT MODES OF DELIVERY IN TWIN PREGNANCIES WITH DIFFERENT PRESENTATIONS	*Phillip C. Greig, Jean-Claude Veille, Linda Henderson* Bowman Gray School of Medicine Winston-Salem, NC
3:15-3:30pm	32	THE EFFECT OF OPERATIVE VAGINAL DELIVERY ON COGNITIVE DEVELOPMENT	*Barbara Wesley, B. Van den Berg, E.A. Reece* Temple School of Medicine Philadelphia, PA and University of California at Berkeley Berkeley, CA

3:30-3:45pm	33	PERINATAL TRANSMISSION OF HEPATITIS C VIRUS	*Enid Leikin, J. Reinus, H. Alter, S. Piazza, J. Shih, B. Jett* New York Medical College, Valhalla, NY and Albert Einstein College of Medicine, Bronx, NY and National Institutes of Health, Bethesda, MD
3:45-4:00pm	34	SECOND TRIMESTER OBSTETRICAL ULTRASOUND IN THE PRENATAL DETECTION OF CONGENITAL HEART DISEASE	*Janet N. Scheel, Nancy A. Callan, Gail D. Pearson, Jean S. Kan, Catherine A. Neill* The Johns Hopkins School of Medicine Baltimore, MD

Saturday, February 8, 1992

8:00am-10:30am	**Oral Session V: Infectious Disease; Maternal/ Fetal Physiology** **Moderator:** ***Frank C. Miller, M.D.*** ***Past President, SPO***	**Grand Salons V-VIII**

8:00-8:15am	35	IS BACTERIAL ENDOTOXIN A CAUSE OF MECONIUM PASSAGE IN UTERO?	*Roberto Romero, M. Mazor, W. Sepulveda, F. Brandt, R. Gonzalez, M. Ramirez, E. Behnke* Yale University, New Haven, CT and Soroka Medical Center/Ben Gurion University, Haifa, Israel and Sotero del Rio Hospital, Santiago, Chile
8:15-8:30am	36	PLACENTA NATURAL KILLER CELL CYTOTOXICITY (NKC) IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PARTURIENTS	*Bernard Gonik, L. Loo, J. Reuben, T. Cowles, A. Helfgott, A. Harris, M. Doyle* University of Texas Medical School and M.D. Anderson Cancer Center Houston, TX
8:30-8:45am	37	AMNIOTIC FLUID INFECTION (AFI) AND PRETERM LABOR IN RHESUS MACAQUES	*Michael G. Gravett, G.J. Haluska, J.L. Edwards, M.J. Cook, S. Baggia, S.S. Witkin, M.J. Novy* Oregon Health Sciences University and Oregon Regional Primate Research Center, Portland, OR and Cornell University, Cornell, NY
8:45-9:00am	38	DOES THE RISK OF PERINATAL TRANSMISSION OF HIV-1 INCREASE WITH SUBSEQUENT PREGNANCIES?	*R.R. Viscarello, N.J. DeGennaro, Y.G. Gollin, W.A. Andiman, J.C. Hobbins* Yale University New Haven, CT

9:00-9:15am	**39**	**HYPOXIC ACIDEMIA DECREASES LEFT VENTRICULAR END-SYSTOLIC ELASTANCE IN FETAL SHEEP**	*R.M. Lewinsky, R.S. Szwarc, L.N. Benson, J.W.K. Ritchie* University of Toronto Toronto, Ontario, Canada
9:15-9:30am	**40**	**DO ABNORMAL STARLING'S FORCES CAUSE FETAL HYDROPS IN RED CELL ALLOIMMUNIZATION?**	*Kenneth J. Moise, Jr., Robert J. Carpenter, Jr., Diane Hesketh* Baylor College of Medicine Houston, TX
9:30-9:45am	**41**	**LONGITUDINAL CHANGES IN BASAL HEPATIC GLUCOSE PRODUCTION AND SUPPRESSION DURING INSULIN INFUSION IN NORMAL PREGNANT WOMEN**	*Patrick M. Catalano, R.R. Wolfe, E.D. Tyzbir, N. Roman, S. Amini, E.A.H. Sims* University of Vermont, Burlington, VT and Case Western Reserve University, Cleveland, OH and Shriners Burn Institute at University of Texas, Galveston, TX
9:45-10:00am	**42**	**ENDOTHELIUM-DERIVED RELAXING FACTOR MEDIATES ESTROGEN-INDUCED INCREASES IN UTERINE BLOOD FLOW**	*G.A. Van Buren, D-S Yang, T. Siddiqi, K.E. Clark* University of Cincinnati Cincinnati, OH
10:00-10:15am	**43**	**TUMOR NECROSIS FACTOR ALPHA (TNF-α) IN SECOND TRIMESTER AMNIOTIC FLUID IS ASSOCIATED WITH IMPAIRED INTRAUTERINE FETAL GROWTH**	*Kent Heyborne, J. McGregor, S. Witkin, G. Henry* University of Colorado, Denver, Cornell Medical Center, NY and Reproductive Genetics Center, Denver, CO
10:15-10:30am	**44**	**COCAINE DIRECTLY AFFECTS SIGNAL TRANSDUCTION IN HUMAN MYOMETRIAL CELLS**	*Frank Hertelendy, M. Molnar* St. Louis University Medical Center St. Louis, MO

Oral Session I

Prematurity; Labor; Neonatology

Thursday, February 6, 1992
8:00-10:30 a.m.

Moderator: *Thomas J. Garite, M.D.*
President
Society of Perinatal Obstetricians

Grand Salons V-VIII



1 ONCOFETAL FIBRONECTIN IN PATIENTS AT INCREASED RISK FOR PRETERM DELIVERY

Nageotte MP, Hollenbach KA, Vanderwahl BA, Hutch KM
Long Beach Memorial Womens Hospital
University of California, Irvine

In an effort to evaluate oncofetal fibronectin (fFN) as a screening test for premature birth, asymptomatic high risk patients were identified and followed prospectively. Risk factors included multiple gestation, previous preterm labor, previous preterm birth, congenital uterine anomaly and incompetent cervix with cerclage. Beginning at 20 weeks of gestation and repeated weeky until delivery or 37 weeks, a home visiting nurse obtained specimens of cervicovaginal fluid from the posterior fornix. A total of 1144 specimens were obtained (mean 11.2/patient). Specimens were batched and an ELISA immunoassay for fFN was performed following delivery. A specimen was defined as positive when greater than 50 ng fFN/ml was present. Sixty-four patients delivered at or beyond 37 weeks of gestation while 38 patients delivered before 37 completed weeks. However, 6 of these preterm deliveries were induced with a resultant spontaneous preterm birth rate of 33.3% (32/96). By 34 weeks of gestation 16 patients had delivered, only 1 of whom was induced (14.9%, 15/101). As a marker for delivery prior to 37 weeks, fFN had a sensitivity of 90.6%, a specificity of 44.3%, a positive predictive value of 42.6% and a negative predictive value of 91.2% (OR-6.2; 95% CI 1.6-22.4; p = 0.005; RR-4.0; 95% CI 1.5-9.5). For delivery prior to 34 weeks, sensitivity was 93.8%, specificity 58.8%, positive predictive value 30.0% and negative predictive value 98.0% (OR 21.4; 95% CI 2.7-114.4; p = 0.0003; RR-15.3; 95% CI 3.4-68.2).

Conclusion: Oncofetal fibronectin is an excellent marker for preterm delivery in asymptomatic high risk patients.

2 NATURAL INTERLEUKIN-1 RECEPTOR ANTAGONIST BLOCKS INTERLEUKIN-1-INDUCED PROSTAGLANDIN PRODUCTION BY HUMAN INTRAUTERINE TISSUES: THE BASIS FOR A NOVEL APPROACH TO THE TREATMENT OF PRETERM LABOR IN THE SETTING OF INFECTION.

R.Romero, W.Sepulveda,* M.Mazor, C.Dinarello, M Mitchell, Depts. of Ob-Gyn, Yale Univ. Sch. of Med., New Haven, CT; Wayne State Univ., Detroit, MI; The Univ. of Utah, Salt Lake City, UT and Dept. of Geographic Med., Tufts Univ., Boston, MA

Interleukin-1 (IL-1) has been implicated in the mechanisms responsible for preterm labor (PTL) in the setting of infection. Recently, a natural IL-1 receptor antagonist protein (IRAP) has been identified. This new member of the IL-1 gene family seems to have evolved to regulate the biological effects of IL-1α and IL-1β (Proc Natl Acad Sci 1991;88:5232). Inhibition of IL-1-induced prostaglandin (PG) production by intrauterine tissues may have potential value in the treatment of PTL associated with infection. The purpose of these studies was 1) to determine whether IRAP is present in the amniotic fluid (AF) of women with term labor (TL) and PTL (with and without infection) and 2) to study the effects of IRAP on IL-1-induced PG biosynthesis by human amnion and chorion. Materials and Methods: AF was obtained from women with TL and PTL (n = 98). Fluid was cultured for aerobic and anaerobic bacteria and Mycoplasmas. IL-1α, IL-1β and IRAP AF concentrations were measured by immunoassays previously validated for human AF. The effect of IRAP on IL-1-induced PG production by amnion and chorion was studied using primary cultures. Cells were incubated with IRAP and IL-1β for 16 hrs. PGE_2 released into the media was assayed by immunoassay. Results: 1) IRAP was present in all AF samples. 2) AF contains the highest IRAP concentrations detected in any biological fluid to date. 3) AF IRAP concentrations were not increased in women with preterm labor and intraamniotic infection despite dramatically elevated concentrations of IL-1α and IL-1β in the same fluid (PTL with negative AF culture: median=22 ng/ml, range=1.6-70 vs. PTL with positive AF culture: median=38 ng/ml, range=6-70; p >0.05). 4) IRAP blocked IL-1β-induced PGE_2 production by amnion and chorion in a dose-dependent manner. 5) IRAP, by itself, did not stimulate PGE_2 release by amnion and chorion when used in concentrations ranging from 0.1 mg/ml to 1000 ng/ml. Conclusion: 1) IRAP is a physiologic component of AF; 2) The release of IL-1α and IL-1β into the AF in women with premature labor is not associated with changes in IRAP bioavailability in AF; 3) IRAP blocks IL-1-induced FG production by amnion and chorion; 4) Anti-cytokine agents may be of value in the treatment of PTL.

3 KETOROLAC BLOCKS RITODRINE-STIMULATED PRODUCTION OF $PGF_{2\alpha}$ IN PREGNANT SHEEP.

Phillip N. Rauk, Steven A. Laifer, University of Pittsburgh School of Medicine, Magee-Womens Hospital, Pittsburgh, PA

We have previously demonstrated that ritodrine infusion to pregnant sheep increases uteroplacental production of prostaglandin (PG)$F_{2\alpha}$. We have speculated that the increase in uterotonic $PGF_{2\alpha}$ may contribute to the tachyphylaxis that occurs with ritodrine. We performed the following study to determine if infusion of the prostaglandin synthesis inhibitor, ketorolac, would block ritodrine-induced production of $PGF_{2\alpha}$, when the 2 agents are administered in combination to pregnant sheep. In 5 pregnant sheep (gestational ages 110-120/147), we placed catheters in the aorta, vena cava and in the uterine vein from the pregnant uterine horn. In random order on different days we infused saline, ritodrine (4 μg/kg/min), ketorolac (1.2 μg/kg/min), or a combination of ritodrine and ketorolac, into the venous catheter at a rate of 10 cc/min over a 4h period. Uterine venous and maternal arterial blood was sampled 60 min before and immediately before the infusion, and then at 60, 120, 180, 240 min during the infusion and assayed for $PGF_{2\alpha}$. Ritodrine significantly increased uterine venous $PGF_{2\alpha}$ during the 4h infusion (mean increase at 4h 1.16 ng/ml, $p<0.05$). When ritodrine and ketorolac were administered in combination, there was no change in uterine venous $PGF_{2\alpha}$ throughout the 4h infusion. Ketorolac completely blocked the ritodrine-induced production of $PGF_{2\alpha}$. This is the first study to show that a prostaglandin synthesis inhibitor can effectively block uteroplacental prostaglandin production stimulated by ritodrine in vivo. Based on these results, there appears to be definite physiologic advantage for tocolytic regimens that include a combination of ritodrine and a prostaglandin synthesis inhibitor.

4 CESAREAN SECTION FOR FETAL INDICATIONS AT THE LIMITS OF FETAL VIABILITY (1986 TO 1991).

Erol Amon,MD, Sam Moyn* St. Louis University, St. Mary's Health Center, Department of OB-GYN.

We repeated a similar survey of the SPO membership to assess changes in management regarding cesarean delivery at the limits of viability. Data on 560, 1991 members, were compared to 404, 1986 members. 85% of respondents are attendings in MFM. 30% are strongly influenced by legal concerns. 70% rely more on GA than EFW for accuracy in deciding for C/S. 60% are Univ. based; 24% Univ. affil.; and 16% non-Univ. The lower limit for initiation of cesarean delivery for fetal indications are summarized in the table.

	% Response (cumulative %)			
Lowest GA to	C/S-fetal distress		C/S-breech	
initiate mgmt	1986	1991	1986	1991
22(weeks)	0	0.5	0	0.2
23	0.4	3.4(4)	0	2.7(3)
24	18(18)	33(37)	14	24(27)
25	25(43)	35(72)	22(36)	29(56)
26	47(90)	27(99)	46(82)	27(83)
27	8(98)	1(100)	7(89)	4(87)
28	2(100)	0.4	4(93)	2(89)
>28	-	-	2(95)	1(90)
Do not usually C/S for breech			5%	10%

Although individualization of care prevails, the cumulative percentage of members willing to perform C/S at 24 weeks gestation for fetal indications has doubled during the last five years.

5 NEONATAL INTRAVENTRICULAR HEMORRHAGE (IVH) FOLLOWING MATERNAL BETA-SYMPATHOMIMETIC TOCOLYSIS.

LJ Groome, RL Goldenberg, SP Cliver,* RO Davis, RL Copper,*
University of Alabama Hospitals, Birmingham, Alabama

There has not been a reduction in neonatal morbidity or mortality associated with the widespread use of ß-mimetic agents (BMA) to delay delivery. An increase in the incidence of IVH following ß-mimetic tocolysis may be partly responsible for this lack of improvement even if delivery is delayed. This study was designed to determine if the incidence of IVH is increased in the offspring of women who received a BMA for preterm labor tocolysis. The population consisted of women who delivered singleton liveborn infants free of neurological anomalies at 25-36 weeks during a multicenter preterm birth prevention trial (1982-86). Based on factors such as gestational age and cervical dilatation, women in preterm labor were either not treated, given magnesium sulfate ($MgSO_4$) or received a BMA. IVH was diagnosed by ultrasound, routinely performed on infants <1500 gms and as indicated at higher weights. Of the 1978 infants with either spontaneous preterm labor or PROM who delivered preterm, 105 (5.3%) had IVH and 32 (1.6%) had grade III or IV IVH. Use of a BMA was associated with a 4-fold increase in the incidence of IVH compared to the use of either $MgSO_4$ or no tocolytic agent ($p<0.001$). In virtually all preterm gestational age groups, there was a 2-fold and generally significant increase in the incidence of IVH following BMA tocolysis. A logistic regression analysis was performed adjusting for the type of tocolytic agent, gestational age at delivery, birthweight, medical center, route of delivery, indication for delivery, race, infant sex, and respiratory distress syndrome. The use of a BMA was found to be significantly associated with IVH [Odds Ratio (OR) of 2.3 (1.23-4.29)]. In addition, BMA tocolysis was associated with a significant increase in the incidence of grades III and IV IVH when compared to no treatment [OR of 2.91 (1.06-7.97)]. This retrospective study suggests that BMA tocolytic therapy may be associated with at least a 2-fold increase in the incidence of IVH even when other risk factors are taken into account.

6 THE CANADIAN MULTICENTRE RCT OF EARLY AMNIOTOMY.

WD Fraser[X], S Marcoux[X], JM Moutquin, A Christen[X], BA Armson, JP Verreault[X], N Okun[X], C Nimrod, AK Joshi[X], H Cohen, L Bayer, T Doran[X], P Bernstein, J Carroll[X], S Bottoms, F Galerneau[X], Laval University, Quebec, Canada.

The goal of the study was to determine if a policy of early amniotomy for nulliparae in term spontaneous labour is an effective means to prevent dystocia. In 11 centres, 925 women were randomly allocated to either early amniotomy (ARM) or to conservation of the membranes (COM). For the purpose of the study, dystocia was defined as a period of at least 4 hours, after 3 centimeters dilatation had been achieved, during which the mean rate of cervical dilatation was < 0.5 cm/hr. Treatment groups were similar with respect to age, GA, years of education, weight, cervical status, and baby's birthweight. The median interval from randomization to membrane rupture was 20 min. in the ARM group and 230 min. in the COM group. Epidural anesthesia and electronic fetal monitoring were used with similar frequency in the two groups. Oxytocin was used prior to a diagnosis of dystocia in a similar proportion of women in the two groups (ARM - 13.2%; COM - 11.4%). Dystocia was less frequent in the ARM group than in the COM group (33.6% vs 44.6%; p = 0.001). The mean interval from randomization to delivery for women delivering vaginally was approximately 1.5 hours shorter in the ARM than in the COM group ($p < 0.0001$). The distribution of modes of delivery was similar in the two groups. Groups were also comparable with respect to indicators of neonatal status (APGAR 5 < 7, arterial cord pH < 7.20, admission to ICU, cephalohematoma). Thus, a policy of early amniotomy was found to be protective for dystocia by the study definition (RR = 0.75).

7 EXPRESSION OF PARATHYROID HORMONE-RELATED PEPTIDE (PTHrP) mRNA IN PLACENTAL MEMBRANES AND AMNIOTIC FLUID (AF).

Ferguson II JE, Gorman J[X], Bruns DE[X], Pandian MR[X,+], Bruns MEH[X]. Departments of Obstetrics and Gynecology and Pathology, University of Virginia School of Medicine, Charlottesville, VA, and +Nichols Institute, San Juan Capistrano, CA.

PTHrP was originally discovered in human tumors that produce hypercalcemia of malignancy. The hormone increases cyclic AMP and prostaglandin E_2 in target tissues, but its physiological functions are unknown. We previously reported that PTHrP mRNA is expressed in laboring human uterus. We here report the unexpected finding that PTHrP is abundantly expressed in human amnion and reaches high concentrations in amniotic fluid. PTHrP mRNA was measured by computer-aided densitometry of Northern blot autoradiographs. The hormone was measured by use of a sandwich immunoassay. PTHrP mRNA abundance was 5-15 times that found in myometrium and exceeded that found even in lactating mouse mammary gland, the richest source previously identified. PTHrP mRNA abundance was decreased by 60% ($p<0.025$) in amnion from laboring (n=16) vs. non-laboring (n=16) women. The concentration of PTHrP in AF equaled or exceeded those found in serum of patients with hypercalcemia of malignancy. The mean concentrations of hormone in AF at 16 and 39 weeks were 21 ± 6 and 38 ± 11 pmol/L, respectively. These data suggest that the amnion sustains regulated abundant expression of PTHrP; the high concentrations of PTHrP in AF suggest an important role of PTHrP in normal pregnancy.

8 COMPARISON OF INDUCTION METHODS FOR PREMATURE RUPTURE OF MEMBRANES AT TERM.

J.F. McCaul, L.M. Williams,[X] R.W. Martin, E.F. Magann,[X] L. Gallagher,[X] J.C. Morrison, Dept. Ob/Gyn, Univ. Mississippi Med. Ctr., Jackson, MS

Objective: Determine which method of induction is most beneficial for women at term with premature rupture of the membranes (ROM). Patient Population: Women (n = 96) 36-42 weeks' gestation with documented ROM (< 6 hours, without labor or infection) were given informed consent and randomized to one of three groups. Interventions: Expectant management (E) patients were observed for labor. Oxytocin (O) induction was used in the second group while PGE_2 gel (PG) was given (4-mg dose every 6 hours) to patients in the third group. PG was repeated only in women who were not in active labor. Main Outcome Measures: Rupture to delivery interval, length of labor, maternal infectious complications, incidence of cesarean section, hospital stay, and neonatal outcome parameters. Results: There were no significant differences in cervical exam on admission, length of labor, number of vaginal exams, infectious morbidity (maternal/neonatal), or Apgar scores between the three groups.

Group	N	Duration ROM (d)	Fetal Bradycardia	Maternal Hospital Stay
E	31	1.45	4	3.6 ± 1.4
O	25	.76	0	2.6 ± 0.7
PG	35	.89	1	2.5 ± 1.0

The length of hospital stay was significantly longer in E versus O and PG (P = .02) as was duration of ROM for E patients when compared to the other two groups (P = .01). There were no significant differences in the rate of cesarean birth and while neonatal morbidity was not different between the two groups, there was a significant increase in the number of patients with fetal bradycardia in E versus the other two groups (P = .04). Conclusion: E management of ROM at or near term significantly prolongs hospital stay without the benefit of decreasing abdominal delivery rates and with an increased risk of fetal bradycardia.

9 **DOES ANTENATAL MATERNAL BETAMETHASONE ADMINISTRATION REDUCE NEONATAL MORBIDITY FOLLOWING IMMEDIATE SURFACTANT THERAPY AT DELIVERY?**

Andrew M. Davey*, David M. Sherer, Jacques S. Abramowicz, Christopher Cox*, James W. Kendig*.
University of Rochester, Rochester, New York

Retrospective analysis was performed on the neonatal outcome of 190 premature infants delivered between 24-29 weeks gestation (inclusive) who received prophylactic calf-lung surfactant extract (CLSE), (90 mg intratracheally) to examine the possible effect of antenatal steroid administration on subsequent neonatal morbidity. All deliveries occurred at two level III referral centers. Two groups of infants were compiled and compared. The mothers of one group (n=66) received antenatal betamethasone, and those of the second group (n=124) did not. Statistical analysis was performed with one-sided Mann-Witney and Chi- Square tests and Kaplan-Maier survival curves. **Results**: Both groups were similar for maternal age, gravidity, parity, gestational age at delivery, incidence and duration of ruptured membranes, incidence of chorioamnionitis, fetal presentation, mode of delivery, and 1 and 5 minute Apgar scores.
Neonatal morbidity was as follows:

Neonatal Morbidity	CLSE	Betamethasone + CLSE	p value
Intraventricular Hemorrhage (Grade 3,4)	38.1%	22.6%	0.02
Alive & Free of O_2 Requirements at 28 days	49 5%	64.5%	0.03
Patent Ductus Arteriosus	44.1%	30.9%	0.09
Days on Ventilator Median ±SD	20±4.4	6±4.7	0.04
Days in Level III NICU Median±SD	33±4.4	12±6.7	0.05
Days to Discharge Home Mecian±SD	7E±3.8	71±3.5	0.09

The incidence of survival, pulmonary interstitial emphysema and pneumothorax was not statistically different between the two groups. **Conclusion**: The above results suggest a beneficial effect of antenatal maternal betamethasone administration on subsequent prophylactic CLSE in reducing neonatal morbidity in premature infants delivered ≤ 29 weeks gestation.

10 UMBILICAL ARTERY CREATINE KINASE BRAIN BAND % PREDICTS MAJOR INTRAVENTRICULAR HEMORRHAGE.

R. Figueroa[x], L. Gonzalez[x], U. Verma, R. Carter[x], I. Argani[x], N. Tejani. NY Med. Coll., Valhalla, NY.

Objective Creatine Kinase brain band (CKBB) would be elevated in the umbilical artery (UA) of newborns (NB) who develop major intraventricular hemorrhage (IVH) & periventricular leucomalacia (PVL). **Study design** 46 NB with birth wt (BW) ≤ 1750g were studied. UA blood was analyzed for acid base & CKBB%. Results of fontanelle scans done on days 1&3 divided the NB into groups: normal (N), minor (G_1+G_2), major (G_3+G_4), & PVL. T-test, Anova, Fisher's test, & regression analysis were used. **Results** UA CKBB% was higher in NB with major IVH (34.2±7.6) compared to N (8.9±2.4), minor (11±2.8), & PVL (14.7±8.5) ($p < .001$). A CKB% of >15 (mean of N ±2 SE) was used to reexamine the study group.

BB%	#	GA	BW(g)	Ap1	Ap5	UApH	Major	PVL
≤15	(33)	29±3	1177±356	5±3	7±3	7.25±.1	2	3
>15	(13)	28±3	1050±358	3±3	5±2	7.23±.1	6	1
		ns	ns	ns	$p<.01$	ns	$p<.001$	ns

Analysis of Ap5 subsets showed that NB with Ap5<7 did not have a higher risk of major IVH inspite of a high CKBB%. **Conclusions** 1) Prevalence of a major IVH: total 19.2%, low Ap5 36%, CKBB% ≤ 15 7%, CKBB% >15 53%. 2) Depression in NB with elevated UA CKBB% in the absence of acidosis may be due to an "insult" which later results in major IVH. 3) PVL seen within 3 days of birth may not be predicted by UA CKBB% because it evolves over 7-14 days.

Oral Session II

Medical Complications of Pregnancy; Hypertensive Disease of Pregnancy

Thursday, February 6, 1992
2:00-4:00 p.m.

Moderator: *Julian T. Parer, M.D., Ph.D.*
Program Chairman
1991 SPO Annual Meeting

Grand Salons V-VIII

11 THRESHOLD VALUES FOR GLUCOSE TOLERANCE TEST (GTT) IN PREGNANCY NEED TO BE MODIFIED. N. Boe,[X] J. Dacus, B. Mercer,[X] K. Schulz, B. Sibai. University of Tennessee, Memphis.

Considerable controversy exists regarding plasma glucose values considered abnormal on glucose tolerance tests (O'Sullivan et al. Sacks et al). We hypothesized that pregnant women with a positive glucola screen and two or more abnormal values (as defined by Sacks et al) on the GTT would have a higher incidence of macrosomia and neonatal complications than women with a negative glucola screen. Materials & Methods: The study group included 123 women with a positive glucola screen who subsequently had a normal GTT by O'Sullivan's criteria (Fasting, 105 mg/dl; 1 hour 190 mg/dl; 2 hours, 165 mg/dl; 3 hours, 145 mg/dl), but who had two or more abnormal values using Sacks' threshold values for the GTT (Fasting, 96 mg/dl; 1 hour, 172 mg/dl; 2 hours 152 mg/dl; and 3 hours, 131 mg/dl). The control group included 102 women who had a negative glucola screen. Results: Maternal and perinatal outcome variables analyzed are summarized below. Although the birthweights in the two groups were not significantly different grossly (3350 vs 3240 grams), a statistically significant increase of 158 grams in the study group birthweights was found after correcting for maternal weight at delivery and gestational age ($p<0.01$). Conclusions: Patients with a positive glucola screen and normal GTT by O'Sullivan's criteria, but abnormal GTT by Sacks' criteria are at increased risk for cesarean section and neonatal morbidity as compared to patients with a negative glucola screen. These findings suggest that the GTT criteria of O'Sullivan should be modified.

Variable	Study Group n=123	Control Group n=102	P value
Maternal weight, at delivery	190±41	177±48	0.03
Delivery by C/S (%)	22.0	9.9	<0.02
LGA (%)	21.1	13.7	0.16
Percent macrosomia (>4000 g)	15	7	0.13
Neonatal hypoglycemia (%)	8	1	<0.02

LGA=large for gestational age, C/S=cesarean section

12 NEONATAL OUTCOME IN PREGNANCIES COMPLICATED BY HYPERTHYROIDISM L. Millar, MD[X], D. Wing, MD[X], P. Koonings, MD[X], M. Montoro, MD[X], J. Mestman, MD[X]
University of Southern California, Los Angeles

A review of 205 patients (1974-1990) was performed to analyze efficacy of treatment and effect of TSH receptor antibody on neonatal outcome. Hyperthyroid patients at delivery had the highest percentage of LBW infants and a 3-fold increase in perinatal mortality and morbidity when compared to euthyroid patients. Euthyroid patients at delivery had an increased incidence of LBW infants if they were hyperthyroid at initial presentation when compared with euthyroid patients at presentation.

STATE AT PRESENTATION	STATE AT DELIVERY	# PATIENTS	# LBW INFANTS	%
Euthyroid	Euthyroid	52	5	10
Hyperthyroid	Euthyroid	81	16	20
Hyperthyroid	Hyperthyroid	44	16	36
	Noncompliant	28	9	32

TSH receptor antibody (TBII and TSI) was evaluated in 95 patients and 38 had positive tests (40%). TBII and TSI levels < 50% regardless of thyroid state were not associated with LBW. Nine patients (8%) had a TBII or TSI >50%. Five of nine had LBW infants (55%) despite medical treatment with a decreasing FT4I and TBII level at delivery. **Thus, a TBII or TSI >50% at presentation is associated with an increased incidence of LBW, even with treatment and decreasing antibody levels. In patients with negative or low levels of TSH receptor antibody, control of hyperthyroidism decreases the incidence of LBW. Preconception control of hyperthyroidism further decreases the incidence of LBW.**

13 **ANTIPHOSPHOLIPID SYNDROME. OUTCOME OF TREATED PREGNANCIES: AN UPDATE OF THE UTAH EXPERIENCE.** **Silver RM,[X] Branch DW, Dudley DJ, Scott JR,[X] Dept. Ob/Gyn, Univ. Utah Med. Ctr., Salt Lake City, UT 84132**

We report 80 consecutive pregnancies in 53 women with antiphospholipid antibodies; 96% had lupus anticoagulant and 98% had IgG anticardiolipin. These patients had 182 previous, untreated pregnancies resulting in 162 losses [80 spontaneous abortions (SAB), 69 fetal deaths (FD), 3 neonatal deaths (ND)] and 22 surviving children (13)%. Fifty met the rigid criteria for antiphospholipid syndrome, and 27 had other autoimmune diseases. In 21 patients with 37 thrombotic events (including 7 strokes) 84% were associated with pregnancy or oral contraceptives. Primary treatments included prednisone (PRED), heparin (HEP), and low-dose aspirin (LDA). Results:

			Surviving Children Delivery		Losses		
Treatment	N	Total	< 32 w	> 32 w	SAB	FD	ND
PRED/LDA	39	21 (54%)	10	11	8*	8	2
HEP/LDA	18	13 (72%)	2	11	1	2	2
PRED/HEP/LDA	12	10 (83%)	3	7	0	1	1
Other	11	7 (64%)	2	5	2	2	0

*** Live embryo not confirmed in all cases.**

Excluding SABs from the analysis yields: 21 (68%) living children in the PRED/LDA group and 13 (76%) in the HEP/LDA group (p=NS). Fifty-four percent of mothers reaching the second trimester developed preclampsia. Fetal distress developed in 51% of cases, and 29% of live borns were SGA. Three patients also had 4 pregnancies complicated by postpartum thrombosis during treatment. Conclusions: (1) There were no significant differences in perinatal outcomes or maternal complications between groups and (2) the high rate of thromboembolic episodes suggests the need for anticoagulation in this population.

14 ACETYLSALICYLIC ACID INHIBITS ANTICARDIOLIPIN ANTIBODY-INDUCED PLATELET-ACTIVATING FACTOR SYNTHESIS. RK Silver, M.D., PD O'Connell,[X] MS Caplan,[X] Departments of OB/GYN and Pediatrics, Northwestern University Medical School, Evanston Hospital, Evanston, Illinois

Anticardiolipin antibodies (ACA) are thought to cause recurrent pregnancy loss by promoting placental and decidual thrombosis. We have shown that endothelial cell platelet-activating factor (PAF) synthesis is enhanced by ACA, and speculate that increased levels of this autacoid in ACA-positive patients, might promote thrombosis via platelet aggregation and fibrin deposition. As acetylsalicylic acid (ASA) has been utilized in these women with the intention of inhibiting platelet aggregation directly (through a reduction in thromboxane A_2 production), we wished to determine if ASA might in addition, influence ACA-mediated endothelial cell PAF synthesis. Endothelial cells were harvested from human umbilical veins, grown to confluence in culture, then incubated for 48 hours with [20%] ACA+, heat-inactivated human serum, plus ASA in a dosage of 1.0 mM/L. After incubation, culture wells were stimulated with 10uM/ml A23187 (a potent PAF agonist). Intracellular PAF was recovered by radiolabeling each monolayer with ^{3}H-acetate, followed by phospholipid extraction and thin-layer chromatography. PAF levels were quantified by averaging triplicate measures for each condition. Results from separate experiments (using a singe ACA+ serum source) are shown below (PAF is expressed as dpm x 10^3):

	Exp 1	Exp 2	Exp 3	Mean (SE)
No ASA	10.6	9.4	8.7	9.6 ± 4.5
ASA	5.9	5.7	2.8	4.8 ± 0.8

A 54% reduction in PAF synthesis was observed in ASA-treated cultures (range of 41-68%; $p=0.016$, two-tailed T-test). In an additional experiment, inhibition of PAF appeared to be dose-dependent, with ASA concentrations of 0, 0.1, 1, 10 and 100mMol/L resulting in PAF levels of 8.7, 3.6, 2.8, 0.5 and 0.1 dpm x 10^3, respectively ($r^2=0.87$; $p=0.022$). As expected, prostacyclin synthesis (measured by its metabolite, 6-keto-F1-alpha) was greatly attenuated in ASA-exposed cultures. These observations suggest that in ACA-positive women, the antithrombotic effects of ASA may relate in part, to reduced endothelial cell PAF synthesis. (Supported by a Dee & Moody Grant, Evanston Hospital)

15 A RANDOMIZED PROSPECTIVE COMPARISON OF NIFEDIPINE AND BED REST VERSUS BED REST ALONE IN THE MANAGEMENT OF PREECLAMPSIA REMOTE FROM TERM, BM Sibai, JR Barton[X], S Akl[X], C Sarinoglu[X], BM Mercer[X], University of Tennessee, Memphis.

Two hundred primigravidas with preeclampsia (hypertension plus proteinuria) at 26-36 weeks' gestation were randomly allocated to be managed with bedrest alone or bedrest plus oral nifedipine 40-120 mg/day. All women had baseline and serial evaluations of maternal and fetal well being. Following hospitalization, patients with adequate blood pressure response and absent proteinuria (<300 mg/24 hr) were subsequently managed on an outpatient basis. There were no differences between the two groups in mean systolic and diastolic blood pressures at time of randomization, however, the nifedipine group had significantly lower systolic ($p<0.0001$) and diastolic ($p< 0.0001$) blood pressures during therapy. There were no differences in number of days of antepartum maternal hospitalization (12.6 ± 7.9 v 12.3 ± 10.3). Table compares the clinical findings in 197 patients (3 were lost to follow-up). The two groups had similar incidences of abruptio placentae (2% v 3%) and HELLP syndrome (2% v 4%). There were no perinatal deaths in either group and no differences in cord gasses between groups. **Conclusions:** Nifedipine therapy for preeclampsia significantly reduces maternal blood pressure but does not shorten maternal hospitalization or improve perinatal outcome.

Mean ± SD	Control (n = 99)	Nifedipine (n = 98)
Admission gest age (wks)	33.5 ± 2.3	32.9 ± 2.6
Pregnancy prolongation (days)	22.3 ± 13.5	22.5 ± 15.7
Delivery for severe HTN # (%)	*18 (18)	9 (9)
Delivered ≥ 37 wk # (%)	60 (60)	50 (51)
Birth weight (grams)	2509 ± 723	2403 ± 769
IUGR # (%)	13 (13)	15 (15)
Cord pH < 7.20	11(13)	9 (10)
Admitted to SCN # (%)	21 (21)	30 (30)

SCN = Special Care Nursery, HTN = hypertension, *P < 0.05

16 MAGNESIUM SULFATE INJECTIONS BLOCK NMDA-INDUCED HIPPOCAMPAL SEIZURES. D.B. Cotton, R.F. Berman,[x] S. Irtenkauf,[x] Dept. Ob/Gyn. Wayne State Univ./Hutzel Hosp., Detroit, MI

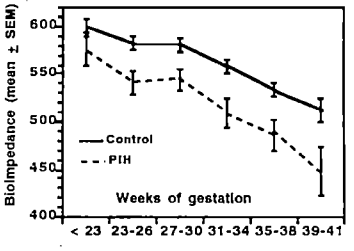
The use of magnesium sulfate in the treatment of seizures and convulsions associated with preeclampsia-eclampsia is well established. However, the mechanism by which magnesium blocks seizures is unknown. Recent evidence has suggested that activation of excitatory amino acid receptors, such as the N-methyl-D-aspartate (NMDA) receptor, can result in seizures and that these receptors are blocked in a voltage dependent manner by magnesium. Thus magnesium sulfate injections may inhibit seizures directly via a magnesium-induced block of NMDA receptors. As a test of this hypothesis, we examined the ability of intraperitoneal injections of magnesium sulfate to block seizures produced by direct injections of 20 μg of NMDA into the dorsal hippocampus of rats, a region enriched in NMDA receptors. NMDA injections into the hippocampus resulted in almost immediate epileptiform activity, with an average onset latency of 40.7 ± 11 sec. and an average initial seizure duration of 19.5 ± 5 sec. These seizures gradually increased in severity and frequency, evolving into status-type seizures after approximately 10-15 min. Animals pretreated with 270 mg/kg magnesium sulfate either failed to seize or showed seizures of greatly reduced severity and duration. Of those magnesium treated animals which did seize, average seizure onset following NMDA was increased to 96 ± 13 sec. and the average duration was reduced to 8.7 ± 4 sec. These data indicate that magnesium can exert marked anticonvulsant effects against NMDA-induced seizures and suggest a possible link between excitatory amino acid transmitters (e.g., NMDA) in the nervous system and seizures associated with preeclampsia-eclampsia.

17 **MAGNESIUM PIDOLATE INFUSION REDUCES ANGIOTENSIN II PRESSOR RESPONSE IN PREGNANT WOMEN.** Tranquilli AL,[X] Mariani ML,[X] Garzetti CG,[X] Valensie H,[X] Romanini C.[X] University of Ancona, Italy

Circulating eicosanoids and intracellular free calcium (Cai) may contribute in the systemic pressor response to infused angiotensin II (AII) in pregnancy. Since magnesium (Mg) may interfere with both those factors, we studied Cai and the pressor response to AII in 10 primigravid women (28-32 weeks' gestation) before and after the infusion of 1 gt Mg pidolate. After the effective pressore dose (EPD) (i.e. the AII infusion rate at which the diastolic blood pressure increased 20 mmHg) was achieved, or a maximum of 64 ng/kg/min rate was reached, we infused 1 gr Mg pidolate and repeated the test. Cai was measured by means of fluorescent probes at the beginning and the end of both tests. Six women were classified as refractory to AII and 4 as sensitive (EPD<10 ng/kg/min). After Mg pidolate infusion, the 4 sensitive women became refractory, and EPD was significantly enhanced in 3 out of the 6 refractory. Cai increased significantly during AII infusion, whereas, after Mg pidolate administration, it did not change. From these results, Mg pidolate may therefore enhance the vascular refractoriness, and Cai mediate the pressor response to AII, in pregnancy.

18 **A PROSPECTIVE STUDY OF BIOIMPEDANCE ANALYSIS IN NORMAL AND HYPERTENSIVE PREGNANCIES.** TM Goodwin, S Estrada[x], KA Smith[x], L Bernstein[x], R Artal. University of Southern. California, Los Angeles, CA

Bioelectrical impedance analysis (BIA) has been proposed as a safe and inexpensive method of estimating total body water in normal and pathologic states. The formula for the volume of a conductor, $V=L^2/Z$, describes the relationship between bioimpedance (Z), total body water (V) and height (L). A prospective study was undertaken to test the hypothesis that lower bioimpedance (indicative of increased body water) is detectable prior to the time of diagnosis in women destined to develop pregnancy induced hypertension (PIH). Six hundred determinations were made on 161 healthy nulliparae from 18 to 41 weeks gestation. Thirty-six subjects (22%) developed PIH, but only 3 were diagnosed before 36 weeks gestation. Bioimpedance correlated negatively with gestational age among normals($r=.4, p<.001$) and patients who developed PIH ($r=.5, p<.001$). The data are displayed above as means for arbitrarily defined gestational age intervals.



Mean bioimpedance differed significantly between normal and PIH subjects beginning as early as 23 weeks gestation. BIA may be useful in studying the natural history of PIH.

Oral Session III

Genetics and Teratology; Fetal Therapy; Placental Physiology

Friday, February 7, 1992
8:00-10:00 a.m.

Moderator: *Garland D. Anderson, M.D.*
President Elect
Society of Perinatal Obstetricians

Grand Salons V-VIII

19 **A PROSPECTIVE EVALUATION OF TRIPLE MARKER MATERNAL SERUM SCREENING FOR TRISOMY-21.** EY Cheng,[X] DA Luthy, DE Hickok, R Lieppman, RG Resta,[X] M Williams, [X] A Zebelman,[X] F Luthardt,[X] Swedish Hosp. Med. Ctr., Seattle WA

Early data suggest the use of triple marker analysis from maternal serum may be an effective screening tool for the prenatal diagnosis of Trisomy-21. From 1/1/90 to 8/15/91 we evaluated the triple marker screen obtained at 16-18 weeks gestation in singleton, non-diabetic pregnancies, using MSAFP (HYBERTECH), unconjugated estriol, and total HCG (AMERSHAM) as the analytes measured. We defined a midtrimester risk for Trisomy-21 of ≤1:195 as a positive screen. Pilot studies indicated that 7% of pregnancies would be screen-positive and approximately 2/3 of all cases of Trisomy-21 could be detected. During the 20-month study period 7785 pregnancies were tested with a mean maternal age of 29.3±4.8. 572 pregnancies (7.5%) were screen-positive. 298 of the 7785 pregnancies screened (3.8%) eventually underwent amniocentesis, yielding 21 cases of Trisomy-21. Twenty-one of 298 of amniocenteses (PVP=7%) resulted in a diagnosis of Trisomy-21, comparing favorably to amniocenteses for advanced maternal age, in which 1-2% of procedures yield Trisomy-21. The use of 3 markers (MSAFP, HCG, estriol) improved screening performance, compared to MSAFP and HCG without estriol. The data suggest triple marker analysis is an effective prenatal screen for Trisomy-21.

20 MID-TRIMESTER ECHOGENIC BOWEL AND CHROMOSOMAL ABNORMALITIES. AL Scioscia, D Pretorius[X], N Budorick[X], T Cahill[X], F Axelrod[X], G Leopold[X]. University of California, San Diego, La Jolla, CA.

Sonographic detection of echogenic bowel (EB) in the midtrimester fetus has been associated with cystic fibrosis (CF) and aneuploidy, as well as being a normal variant. From 8/1/90-7/31/91, 22 cases of EB were prospectively detected. Sonograms were performed for the following indications: advanced maternal age(8),↑MSAFP(6),↓MSAFP(4), anatomic survey(2), and outside studies revealing EB (1) and polyhydramnios and EB (1). Gestational age ranged from 15-26 wks, mean 18 wks. Families were counselled regarding the association of EB with CF and aneuploidy and offered testing; 19 amniocenteses were performed, and 17 chose DNA-based CF risk assessment. Six trisomic fetuses were detected (Trisomy 21 (5) Trisomy 18 (1)). No fetus with CF was detected. The diagnosis of EB can be subjective due to technical variability. Equipment, settings and maternal habitus all affect bowel appearance. To determine reliability and interobserver variability, the 22 cases and 10 randomly selected controls were reviewed by 4 authors and graded as: normal-0, mild-1, or bright-2. All agreed on grade (Gr) in 15 cases, 3 of 4 agreed in 14, and in 3 cases the assessment was split. In only one case was the disagreement greater than 1 Gr. To further analyze the 22 cases of EB, Gr was assigned by consensus, 10 cases were Gr 2 and 10 cases Gr 1. Two cases were split between mild and bright and arbitrarily assigned Gr 2, both had normal studies. Of the 12 cases with Gr 2 EB - 5 trisomic fetuses were detected; 1 trisomic fetus was detected in the 10 with Gr 1 EB. Other abnormalities detected prenatally in the trisomic fetuses included - Trisomy 18 -VSD, absent stomach, club feet, clenched hands and renal abnormalities; Trisomy 21-nuchal thickening (NT) of 5.7 mm (1), NT 5.1 mm and short femur (1), bilateral choroid plexus cysts and NT 4 mm (1); no abnormalities detected (2). In 3 trisomic fetuses, pathologic examination of the bowel revealed no gross or microscopic abnormalities. CF studies revealed no parent or fetus with the delta F508 mutation and the haplotype distribution was unremarkable. A larger series is necessary to determine whether CF testing is efficacious. Conclusion:(1) It appears that interobserver error is sufficiently small to allow detection of EB. (2) Six of 22 fetuses with EB proved to be trisomic; those with brightly EB were at greatest risk.

21 PREGNANCY LOSS AFTER FIRST TRIMESTER ULTRASONOGRAPHIC DOCUMENTATION OF EMBRYONIC/FETAL CARDIAC ACTIVITY. Jeffrey M. Barrett, M.D., Jennifer Brinson, R.N.C.[X], Watson Clinic, Lakeland, Florida.

A prospective study was performed to evaluate the characteristics of pregnancy loss before 26 weeks when there is first trimester documentation of embryonic/fetal cardiac activity. Pregnancy dating and documentation of cardiac activity was performed with real time ultrasonography. Pregnancy losses were evaluated and dated with ultrasonography and/or pathologic macroscopic evaluation of most cases. A total of 897 consecutive patients were evaluated with pregnancy outcome known in 99.8%. The overall loss rate after documentation of embryonic/fetal cardiac activity, corrected for elective first trimester termination, was 4.6%, increasing from 10.5% <7 weeks to 2.2% >/10 weeks and 1.6% >/15 weeks. Over half of the losses occurred before 10 weeks, with there being no relationship between maternal age and frequency of loss in this group. However, with gestational age >/10 weeks, maternal age was a factor in the frequency of pregnancy loss with the total loss rate 1.6% <age 35 and 5.4% >/age 35. The age related increase in pregnancy loss continued to be present when corrected for pregnancies terminated due to chromosomal or severe structural abnormalities. These findings have important implications regarding the safety of invasive fetal testing and the evaluation of exogenous factors which may cause fetal loss.

22 EVALUATION OF FETAL BLOOD CONTENT IN TRANSABDOMINAL AND TRANSCERVICAL CHORIONIC VILLUS SAMPLES. K. Blakemore, I. Baser[X], N. Callan, R.S. Shirey[X], T. Kickler[X], M. Blitzer[X]. The Johns Hopkins Univ. Sch. of Med. and Univ. of Maryland, Balto., MD.

The risk of fetal blood loss with first trimester chorionic villus sampling (CVS) has attained renewed importance. Fetal limb reduction abnormalities that appear consistent with a vascular disruptive etiology have raised questions as to whether or not CVS is potentially teratogenic, or whether the technique employed, transcervical (TC) or transabdominal (TA), matters. To determine how often fetal blood is actually retrieved with CVS, we examined 70 first trimester CVS aspirates, i.e. the blood surrounding the villus tissue, by acid-elution staining for fetal hemoglobin (HbF). Forty aspirates were obtained by TC catheter aspiration, and 30 by TA aspiration using a 20 gauge spinal needle. In 23 cases, pre- and post-procedure maternal serum alpha fetoprotein (MSAFP) levels were obtained (16 TC and 7 TA). The percentage of HbF positive cells was ≥10% in 45/70 aspirates (64%), ≥30% in 25/70 aspirates (36%), and ≥80% in 8 cases (11%). All 8 aspirates ≥80% HbF were obtained transabdominally. Chi square analysis of TA vs TC aspirates revealed TA aspirates to have a consistently higher %HbF ($p<.02$,.005, and .001 respectively). Mean %HbF, TA=43% vs TC=16%; was also statistically different ($p<.001$). The mean sample size (mg of villi) differed between the two groups (TA=16 mg vs TC=29 mg; $p<.001$) with TC aspirates appearing more bloody on a scale of 0-4. The TA group had a greater proportion of patients whose MSAFP values increased by >50%, but this did not reach statistical significance with these small patient numbers. These data suggest that TA CVS may be associated with greater fetal blood spillage; however, the acid elution technique cannot directly quantify fetal blood amount, as it varies with the amount of maternal blood present. Our data do provide direct evidence that the integrity of the placenta's fetal vasculature is disrupted to at least some degree in the majority of CVS procedures, both TA and TC. A larger study including AFP measurement on CVS aspirates is underway.

23 DIAGNOSIS AND TREATMENT OF TWIN TO TWIN TRANSFUSION SYNDROME (TTTS). C. Weiner and A. Ludomirsky, Univ Ia Hosp and Penn Hosp, Iowa City, Ia 52242

Authors investigating the "stuck" twin have applied such therapies as laser ablation, therapeutic amniocenteses, and digoxin. In each, TTTs was assumed but not objectively documented We report 20 pregnancies with acute hydramnios and a "stuck" twin at 23.8±2w (range 21-27w) where TTTs was ***documented antenatally***. This represents < ½ of all "stuck" twins evaluated Cordocenteses demonstrated anemia in each "stuck" twin and polycythemia in each larger twin. In 6, adult RBCs were infused to the donor and later identified in the recipient. 15/20 presented <25 w. All patients had serial, therapeutic amniocenteses (>1000 ml per episode), one a pregnancy termination, three a selective fetocide (donor twin), and 3 partial exchange transfusions (PET) for either anemia or polycythemia. Despite aggressive therapy, the delivery GA was 28.6 ±4w and only 17/40 (43%) fetuses survived (<25w at presentation, 10/30; ≥25w 7/10). ***Anemia/polycythemia persisted when therapeutic amniocentesis was the sole treatment.***

	Hct	TotPro	Alb	Hydrops	Survived
Recipients	49±5	5.5±1	3.0±.3	6/20	7/20
Donors	27±5	2.7±.6	1.3±.2	0/20	10/20

Hyperviscosity resulted from both polycythemia and hyperproteinemia. All recipients had Tot Pro and Alb above the 97.5 centile. Hydrops was observed only <25w. Hydropic fetuses treated by PET had a high preload (elevated umbilical venous pressure, UVP) which was acutely lowered. In the two instances where 0.9NS was used for the PET, the UVP declined but the hydrops worsened. Plasmanate maintained the COP in one fetus and the hydrops resolved. **CONCLUSIONS:** ***1) a minority of "stuck" twins result from TTTs; 2) the earlier in GA TTTs manifests, the worse the prognosis; 3) serial therapeutic amniocentesis prolongs gestation, but there is no evidence it alters the dynamics of TTTs; 4) hyperviscosity results from polycythemia and hyperproteinemia; 6) PET may reverse hydrops if the COP is unaltered.***

24 LYMPHOCYTE SUBSETS IN PRENATALLY OBTAINED FETAL BLOOD. SM Berry, J Kaplan[X], NL Fine[X], JA Bichalski[X], MP Dombrowski, NB Isada, MI Evans, DB Cotton. Departments of Ob/Gyn & Pediatrics Wayne State University/Hutzel Hospital & Children's Hospital of Michigan, Detroit, MI.

The diagnosis of fetal viral or protozoal infection is complicated by the inability of the fetus to produce IgM antibody until the late second trimester, and the difficulty of culturing the organisms. "Nonspecific" markers of infection such as thrombocytopenia, eosinophilia, anemia, and elevations in liver function tests have been used to diagnose fetal infection in the absence of traditional serologic markers. Because infection is known to induce changes in lymphocyte subsets, we began using flow cytometry on fetal blood to establish baseline values for the proportions of peripheral blood lymphocytes expressing a variety of cell surface markers. The specimens were obtained by cordocentesis at different gestational ages (GA) for a variety of indications. Two-color flow cytometric analysis was performed on cells from 42 cordocentesis specimens, (GA=19-39), 16 umbilical cord specimens obtained at delivery, and 30 normal adult controls. None of the specimens showed specific or nonspecific evidence of infection. The cell surface markers examined included CD3, CD4, CD5, CD20, CD38, CD56, and CD57. No significant differences between the three groups were found for CD4, CD8, or CD20. Compared to adult lymphocytes, fetal and umbilical cord lymphocytes showed slight reductions in %CD5 ($p < 0.05$), marked reductions in %CD57 ($p < 0.05$), and consistent increases in %CD5+CD20+ ($p < 0.05$). The most striking differences observed were marked increases in CD38+ ($p < 0.05$) cells in fetal and cord blood. This was primarily due to increases in the proportion of CD3+CD38+ cells which probably represent proliferating T cells. Umbilical cord lymphocytes had higher proportions of CD3-CD38+ cells than either fetal or adult specimens which may, therefore, reflect a change induced by the "stress of delivery". These results should form the basis for future assessment of the value of lymphocyte marker analysis for detection of fetal infection.

25 PRODUCTION OF ENDOTHELIN-1 BY HUMAN TROPHOBLASTS P Samuels, J D Steinfeld[X], M Rhoa[X], S Murray[X], J Amico[X], D B Cines[X], K R McCrae[X]. Depts of Obstetrics & Gynecology, Medicine and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA and University of Pittsburgh, Pittsburgh, PA

Endothelin-1 (ET-1) is a 21 amino acid peptide with potent vasoactive properties produced by endothelial cells, macrophages, and other cell types. Increased plasma concentrations of ET-1 have been detected in some gravidas with preeclampsia and intrauterine growth retardation. However, the plasma concentrations of ET-1 are only modestly elevated in these conditions, and it is likely that ET-1 acts as an autocrine or paracrine mediator of vasospasm. Moreover, increased vascular resistance may begin within the placenta. Since trophoblasts are the most prevalent cell type in the placenta, we investigated whether they produce ET-1. Trophoblasts were digested from normal term placentae using trypsin and DNAse, isolated using a Percoll gradient, and allowed to adhere to fibronectin-coated plastic wells. Cytotrophoblasts comprise >95% of the cell population isolated. Trophoblasts were cultured in Dulbeco's Modified Eagle Medium using 2% Ultroser as a serum substitute, and the concentration of ET-1 in conditioned media was measured using a radioimmunoassay. We measured the production of ET-1 24 (n=27), 48 (n=16), and 72 (n=8) hours after isolation. During the first 24 hours, trophoblasts produced 28 ± 11.5 fmol of ET-1 per 10^6 cells. Trophoblasts continued to synthesize ET-1 over the next two days (20.3 ±10.2 fmol/10^6 cells and 22.8 ± 15.8 fmol/10^6 cells produced between days 1-2 and days 2-3, respectively). Synthesis was confirmed by Northern blot analysis of trophoblast mRNA using endothelin-specific cDNA. Human umbilical vein endothelial cells, used as a positive control, produced 102.5 ± 30.8 fmol/10^6 cells during the first 24 hours in culture. **Conclusion:** Cultured term trophoblasts synthesize endothelin-1. The amount of ET-1 produced by these cells is approximately 25% of that produced by endothelial cells. Since the number of trophoblasts exceeds that of any other cell type in the placenta, production of ET-1 by trophoblasts may contribute to the regulation of vascular tone and participate in the pathogenesis of preeclampsia and related disorders.

26 THE EFFECTS OF LOW-DOSE ASPIRIN ON PROSTACYCLIN AND THROMBOXANE PRODUCTION BY THE PERFUSED HUMAN PLACENTA: Robert L. Jacobson, M.D.[X], Anthony Brewer, B.S.[X], Tariq A. Siddiqi, M.D. and Leslie Myatt, Ph.D.[X], University of Cincinnati Medical Center, Cincinnati, OH USA.

There is evidence for altered fetal-placental blood flow in idiopathic intrauterine growth retardation (IUGR) which is manifested by an abnormal umbilical systolic/diastolic (S/D) blood flow velocity ratio. Both preeclampsia and IUGR are asscociated with reduced prostacyclin (PGI_2) production by both maternal and fetal tissues resulting in a relative dominance of thromboxane A_2 (TXA_2) over PGI_2. Low-dose aspirin, acetyl salicylic acid (ASA) selectively inhibits TXA_2 synthesis and may alter feto-placental blood flow. The purpose of this study was to determine the effect of infusion of low-dose ASA into the maternal circulation of the dually perfused isolated human placental cotyledon on fetal and maternal PGI_2 and TXA_2 production. Human placental cotyledons were perfused with tissue culture medium 199 plus 5% polyvinyl-pyrrolidone gassed with 95% O_2, 5% CO_2 at flow rates of 10 ml/min (maternal) and 4 ml/min (fetal). ASA (10^{-5} mol/L) was added to the maternal circuit and cotyledons were perfused for one hour with aliquots taken from the closed fetal circuit every five minutes. Thromboxane B_2 (TXB_2) and 6-keto prostaglandin $F_{1\alpha}$ (metabolites of TXA_2 and PGI_2 respectively) were assayed by radioimmunoassay. Our data indicate a significant fall in maternal production of 6-keto-$PGF_{1\alpha}$ and maternal TXB_2 which then increased to pre-ASA infusion levels. There was a decrease in fetal 6-keto-$PGF_{1\alpha}$ which returned to pre-ASA infusion levels. Fetal TXB_2 production, however, significantly decreased and remained inhibited during the one-hour perfusion period. Maternal and fetal 6-keto-$PGF_{1\alpha}$/TXB_2 ratios remained unchanged. As TXA_2 has a vasoconstrictive effect on arteriolar smooth muscle, the significant and sustained inhibition of its production in the fetal-placental circulation may be responsible in vivo for the lowering of placental vascular resistance as manifested in vivo by the improved S/D ratio seen on Doppler velocimetry and the improved fetal weight, head circumference and placental weight noted by other authors.

Oral Session IV

Clinical/Operative Obstetrics; Ultrasound; Infectious Disease

Friday, February 7, 1992
2:00-4:00 p.m.

Moderator: *Sze-ya Yeh, M.D.*
Secretary/Treasurer
Society of Perinatal Obstetricians

Grand Salons V-VIII

27 A SIGNIFICANT REDUCTION IN CESAREAN DELIVERIES: EFFECT ON PERINATAL OUTCOME

Luis Sanchez-Ramos, M.D., Mark T. Cullen, M.D., Carol Walker, R.N.[X] Division of Maternal-Fetal Medicine, University of Florida, Jacksonville, FL.

In the United States the rate of cesarean sections has quintupled from 5% of obstetric deliveries in 1964 to approximately 25% in 1988.The increase in cesarean deliveries occurred against a background of better survival rates and lower morbidity rates for babies. Some have assumed that these changes were linked. From 1986 to 1990 we have reduced the cesarean section rate from 27% to 8%. A key question is whether this has been accomplished safely, both for the mother and for her infant. In search for this answer we evaluated perinatal outcome in 25,356 deliveries occurring during this five year period. Perinatal outcome was evaluated by analysis of Apgar scores, cord gases, NICU admissions. perinatal mortality, meconium aspiration, seizures, NICU length of stay, birth asphyxia, and birth trauma. In evaluating neonatal outcome the distribution of birthweights and gestational ages, and the degree of prenatal care were addressed. No differences in perinatal outcome was noted, and in many instances improvement occurred. Our experience suggests that cesarean section rates can be substantially reduced without compromising the newborn.

28 VAGINAL DELIVERY OF THE NON-VERTEX SECOND TWIN

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In order to test the hypothesis, that there is no difference in morbidity or mortality between vertex (vtx) and non-vertex (non/vtx) vaginal delivery of second twins, we reviewed 781 consecutive twin deliveries achieving greater than 20 weeks of gestation occurring at our hospital between January 1, 1985 and December 31,1988. Outcome measures evaluated include: 5 minute Apgars, length of hospital stay, NICU admissions, and neonatal deaths. The medical records were retrospectively reviewed for all live born, vaginally delivered second twins with respect to presentation (vtx-non/vtx) and the above mentioned outcome variables. Statistical analysis was performed using χ^2 and Mann-Whitney U tests. During the period of time encompassed by this study there were 407 vaginally born second twins, 17 of which were stillborn (42/1000). Of the remaining 390, 207 (53.1%) were delivered as vtx, and 183 (46.9%) were non/vtx vaginal deliveries. The vast majority (>95%) of these non/vtx vaginal deliveries were total breech extractions. Results for live born second twins were:

	VTX N=207	NON/VTX N=183
5 minute Apgars<7	11 (5.4%)	13 (7.1%)
NICU admissions	38 (184/1000)	37 (202/1000)
Neonatal deaths	14 (68/1000)	9 (49/1000)
Hospital days-median	3	3
Hospital days-mode	2	2

There were no statistically significant differences for any of the neonatal outcome variables, even when stratified by birthweight. These results support the null hypothesis to a confidence limit of 95%, and substantiate non/vtx vaginal delivery of the second twin as a safe intrapartum management option.

29 PRENATAL CARE: DIFFERENTIAL EFFECTS ON MATERNAL AND NEONATAL OUTCOMES.

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Prenatal care is of great medical and public concern. Despite this, basic attributes of prenatal care remain poorly understood. We evaluated effects of quantified amounts of prenatal care on maternal and neonatal outcomes. All liveborn, singleton births at University Hospital from 7/81 - 6/85 (n=10,359) were studied. Preliminary analyses showed that an increasing number of prenatal visits showed linear increases in mean birthweight, gestational age, and appropriateness of gestational age. Further analyses were performed taking into account pregnancy duration and amount of prenatal care. The percent of prenatal visits (% PNV) was calculated using the actual number of prenatal visits divided by the number of visits recommended by the Institute of Medicine, adjusted for pregnancy duration. Logistic regression and chi-square analyses of relative risks (RR) for adverse outcomes by % PNV were performed. Increasing % PNV had no effect on maternal outcomes. Survival analyses showed that increasing % PNV correlated with increased birthweight, gestational age, and decreased nursery stay (each $p<0.001$, log rank). The 0% PNV (i.e. no care) neonates had significantly ($\alpha = 0.02$) increased risks of prematurity (RR = 4.0), birthweight <1500 g (RR = 5.6), NICU admission (RR = 2.2) and ventilator use (RR = 3.8). Relative risks decreased in a step-wise fashion as % PNV approached $\geq$ 100%. Direct costs of neonatal care were markedly increased with reduced antenatal care. Prenatal care was strongly associated with improved neonatal outcomes and decreased costs in a graded, step-wise fashion.

30 INTRAUTERINE GROWTH RETARDATION: A COMPARISON OF THE 3RD VERSUS 10TH PERCENTILE.

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Fetal growth retardation, defined as a birthweight at or below the 10th percentile for gestational age, is associated with increased perinatal morbidity and mortality. However, it is unclear whether the most severely affected infants ($\leq$ 3rd percentile) are more frequently associated with identifiable maternal medical or obstetric conditions or have a worse neonatal outcome than those at the 4th-10th percentile. Using the multicenter March of Dimes data base, a growth curve was constructed from 31,890 liveborn, singleton pregnancies after excluding chromosomal or major structural anomalies. Gestational age was based on a composite of the best available criteria for each patient (clinical, ultrasound, Dubowitz). We compared demographic variables, maternal medical or obstetric complications, and neonatal outcome among infants at $\leq$ 3rd percentile (n=963) or the 4th-10th percentiles (n=2268) and compared these groups to infants at the 25th-75th percentile (n=16,012). For each group, the mean gestational age was 39 weeks and the percent of infants $\leq$ 34 weeks was similar. When compared to those at the 4th-10th or 25th-75th percentiles infants at $\leq$ 3rd percentile were more frequently associated with maternal nulliparity, preeclampsia, and smoking, but not with maternal diabetes, hemoglobinopathies, or placenta previa. These infants also had significantly more low Apgar scores ($\leq$ 7 at 1 and 5 minutes), fetal distress in labor, intraventricular hemorrhage, hyperbilirubinemia, hypoglycemia, a higher cesarean section rate, and increased mortality ($p \leq .05$). Infants at the 4th-10th percentile were similar to those at the 25th-75th percentile in neonatal morbidity, but had a significantly higher mortality rate and a stronger association with certain maternal risk factors such as smoking, preeclampsia, and placental abruption. In summary, newborns at the $\leq$ 3rd percentile were more frequently associated with a maternal medical or obstetric complications, and had an increased morbidity and mortality when compared to those at the 4th-10th or the 25th-75th percentile. Interventional studies designed to reduce the adverse effects of low birthweight should focus on this subset of patients.

31 **EVALUATION OF DIFFERENT MODES OF DELIVERY IN TWIN PREGNANCIES WITH DIFFERENT PRESENTATIONS.**
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Four hundred and fifty-two twin deliveries occurred at Forsyth Memorial Hospital between January of 1985 and December of 1990. Thirty-two sets were excluded from analysis because of elective repeat cesarean section, extreme prematurity and prenatal fetal demise. We evaluated twins from 25 weeks to term. All deliveries had Apgar scores recorded and 214 sets had umbilical cord gases available for evaluation. The overall cesarean section rate was 45.2%. There were 225 sets of vertex/vertex twins, 68% delivered vaginally. There were 99 sets of vertex/breech presentations, 44% delivered vaginally. We analyzed the individual and joint effects of gestational age and mode of delivery in the different twin presentations using analysis of covariance. There were no statistically significant differences in fetal outcome when Apgar scores and umbilical cord gases were compared in the different age groups between those delivered vaginally and those by cesarean section for any given twin presentation. There were 16 sets of twins with a vertex presentation/transverse lie. In 6, vaginal delivery was attempted. Three required emergent cesarean section for twin B because of cord prolapse or persistent malpresentation. None of these fetuses showed lower Apgar scores or significantly different umbilical cord gases when compared to the first twin or twins undergoing elective cesarean delivery at the same gestational age and position.

Conclusion: Our data does not support routine abdominal delivery in vertex/non-vertex twin pregnancies for any gestational age.

32 **THE EFFECT OF OPERATIVE VAGINAL DELIVERY ON COGNITIVE DEVELOPMENT**
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Forceps deliveries have been implicated in the causation of birth trauma resulting in long term adverse outcomes. Although many of these studies were relatively small containing many confounding variables including lack of control for socioeconomic status (SES) or non-ideal comparison groups, e.g Cesarean sections, such data have led to a decline in forceps use. Despite the resultant change in obstetrical practice, there is no decrease in the prevalence of neurologic sequelae in school age children. The present project was a collaborative and retrospective study between the University of California at Berkeley (Kaiser Foundation Health Plan, San Francisco) and Temple University School of Medicine in Philadelphia. From a database of 20,000 working women who received obstetrical care, 3,590 children were randomly selected for evaluation at age 5 by the Peabody Picture Vocabulary Test and the Raven Standard Progressive Matrices. Children were stratified according to mode of delivery and the data of each subset were further divided into lengths of hours in active labor. The data were controlled for SES status. Children weighing less than 2500 grams or less than 37 weeks gestation, or those with congenital anomalies were excluded. Of the 3590 children, 93% were tested: 1746 delivered vaginally, 1351 delivered by forceps (low and mid) and the remainder (breech and C-section delivery) were excluded from analysis. Standardized IQ scores of children taking these tests were 50 ± 10 (mean ± standard deviation). There was no significant difference in IQ scores between children delivered spontaneously or by forceps. CONCLUSION: These data demonstrate in a relatively large study of school age children, that the method of delivery is not associated with significant alteration of intelligence quotient. In this light, the general association of forceps delivery with adverse neonatal outcomes cannot be supported.

Mean Test Scores: Peabody and Raven
(Maternal Education ≥ High School)

	Spontaneous		Low-Mid Forceps		Mid Forceps	
	N	X̄	N	X̄	N	X̄
PARITY 0	132	52.18	24	52.21	59	51.87
PARITY 1+	807	51.05	17	52.27	29	51.09

33 PERINATAL TRANSMISSION OF HEPATITIS C VIRUS
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Dept. Transfusion Med, NIH, Bethesda, Md

Hepatitis C virus (HCV) infection is asymptomatic in over 75% of cases, particularly in infants and young children. In about 40% of affected individuals the source of HCV infection is unknown; vertical transmission may be responsible for some of these cases. From 7/1/89 to 7/31/91 we prospectively studied vertical transmission of HCV, using an immunoassay (Ortho Diagnostics, Raritan, NJ) to test 743 mothers and their babies for anti-HCV antibody. Serum from anti-HCV+ mothers and cord blood from their babies also was tested for HCV nucleic acid sequences by nested polymerase chain reaction (PCR). Anti-HCV antibody was detected in serum from 30 mothers (4%) and cord blood of all but 1 of their 31 babies. 11 of 18 mothers tested to date and none of their babies were PCR+. 21 of 22 infants evaluated after discharge from the hospital became anti-HCV- by 33 wks; the remaining child was anti-HCV+ when lost to followup at 11 wks. None of the babies with long-term followup (mean 49 wks) has had reappearance of anti-HCV antibody, and PCR remains negative. Although anti-HCV antibody appears to be transferred passively, vertical transmission of HCV is uncommon if it occurs.

34 **SECOND TRIMESTER OBSTETRICAL ULTRASOUND IN THE PRENATAL DETECTION OF CONGENITAL HEART DISEASE.**
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The Divisions of Maternal Fetal Medicine and Pediatric Cardiology. The Johns Hopkins Medical School, Baltimore, MD.

To determine the effectiveness of second trimester obstetrical ultrasound (STUS) in the prenatal diagnosis of congenital cardiac malformations (CCM), we reviewed referrals for fetal echocardiography (FE) after STUS using maternally reported data from a population based study of all infants with CCM born in our geographic area (The Baltimore-Washington Infant Study). During 1987-89, 1063 infants were born with CCM. Of these 602 (56.7%) had STUS. A total of 69 of 1063 (6.5%) had FE, 47 after STUS and 22 without STUS. Of those with CCM, 52.2% (555 of 1,063) had STUS but not FE. The sensitivity of STUS in detecting critical CCM (CCM readily detectable by 4-chamber view) was 12.7%. The number of pregnancies with STUS and FE by critical lesion are shown:

Critical CCM	n	STUS (%)	FE (% STUS)
Endocardial Cushion	87	35 (40.2)	4 (11.4)
Hypoplastic Left Heart	30	17 (56.7)	2 (11.8)
Ebstein's Anomaly	14	8 (57.1)	2 (25.0)
Single Ventricle	10	5 (50.0)	0 (0.0)
Tricuspid Atresia	9	6 (66.7)	1 (16.7)

This suggests, that STUS as practiced during the study period when visualization of the four chamber view was recommended, detected only a small percentage of those with critical CCM. STUS with four chamber view has not been optimally practiced as a screening tool for CCM. Further education of those performing STUS is required to improve prenatal cardiac diagnosis.

Oral Session V

Infectious Disease; Maternal/Fetal Physiology

Saturday, February 8, 1992
8:00-10:30 a.m.

Moderator: *Frank C. Miller, M.D.*
Past President
Society of Perinatal Obstetricians

Grand Salons V-VIII

35 IS BACTERIAL ENDOTOXIN A CAUSE OF MECONIUM PASSAGE IN UTERO?
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The causes of meconium passage during labor are largely unknown. Although hypoxia and acidosis are frequently considered as causes of meconium-stained amniotic fluid (MS-AF), fetal pH and blood gases are within normal range in most cases. Intraamniotic infection has been recently implicated as a cause of MS-AF in preterm labor (AJOG 1991;164:859). No information is available regarding the relationship between the presence of microorganisms and/or their products in AF and meconium passage during term labor. Bacterial endotoxin, a component of the cell wall of Gram-negative bacteria, is a potent bioactive agent that can stimulate gastrointestinal peristalsis and lead to meconium passage in utero. Materials and Methods: A case-control study was designed to compare the detection rate of bacterial endotoxin in clear (n = 88) and MS-AF (n = 88). Endotoxin was assayed with the gel clot limulus amebocyte lysate assay (LAL) using a method previously described (sensitivity = 100 pg/ml) (AJOG 1987;157:815). Results: 1) The rate of positive LAL was greater in MS-AF than in clear AF (44.3% [39/88] vs. 4.5% [4/88]; p <0.001). 2) After heat treatment at 100°C for 4 minutes (a method to inactivate non-endotoxin-cross-reacting substances), 43.5% (17/39) of MS-AF had a repeat positive LAL assay, while only one of four clear AF remained positive. 3) Endotoxin was present more commonly in MS-AF than in clear AF (19.3% [17/88] vs. 3.4% [3/88]; p <0.001). 4) Microorganisms were identified by Gram stain in 12.7% (11/86) of MS-AF and in only 3.5% (3/84) of clear AF (p <0.05). Conclusions: 1) Bacterial endotoxin is frequently present in patients with MS-AF. 2) MS-AF contains a heat-labile substance that cross-reacts with endotoxin in the LAL assay. 3) Intraamniotic infection may be an important and previously unrecognized cause of MS-AF. These findings are novel and have important clinical implications for intrapartum and neonatal management.

36 PLACENTA NATURAL KILLER CELL CYTOTOXICITY (NKC) IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PARTURIENTS.
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HIV disease is characterized by host immune dysfunction and opportunistic infection. Infants born of HIV-infected mothers are at-risk for transplacental acquisition of HIV infection, along with the passage of other potential pathogens into the fetal compartment. To better assess the functional capabilities of the placenta as an immune barrier, we examined placental NKC in 7 HIV-infected and 7 control parturients. Following removal of the maternal decidua, cotyledon-derived tissues were minced and the cells dispersed with Dispase. A Ficoll-paque gradient was used to isolate placental mononuclear cells. In a subset of experiments using a DNA hybridization probe for the Y chromosome, the separated cells were determined to be >75% fetal in origin. NKC was measured using a 4 hour chromium-release assay with labelled K562 target cells at an effector to target cell ratio of 100:1. Clinically, the majority of the HIV-infected subjects were classified as group II by CDC criteria, delivered at or near term (37.6 ± 2.8 weeks), and had infants who weighed significantly less (p<0.04) than the control group (2795 ± 346 gms vs 3302 ± 442 gms). At delivery, all infants were clinically well. Thus far, one infant has died of AIDS, one is culture positive, and the remainder are clinically well. NKC was profoundly depressed in all HIV-associated placentas compared to controls (0.2 ± 0.4% vs 19.3 ± 9.6%, p<0.001). Both adherent and nonadherent cell populations contributed to the measurable NKC-like activity. These are the first data examining the immune capabilities of the placenta in relation to HIV disease. These results demonstrate severely attenuated placental NKC and suggest a loss of placental immune response in otherwise asymptomatic HIV-infected parturients.

37 AMNIOTIC FLUID INFECTION (AFI) AND PRETERM LABOR IN RHESUS MACAQUES.
MG Gravett, GJ Haluska[x], JL Edwards[x], MJ Cook[x], S Baggia[x], SS Witkin[x], MJ Novy. Depts Ob/Gyn OHSU and Cornell, and Oregon Reg Primate Res Ctr, Portland, OR.

To study the relationship between AFI, cytokines, prostaglandins (PG), and preterm labor, experimental AFI was established by intra-amniotic inoculation of 10^6 cfu Group B streptococci in 4 chronically instrumented Rhesus monkeys at 130 days gestation (term is 167 days). Amniotic fluid (AF) was sampled sequentially for bacteria, TNF-α (bioassay), IL-1B (ELISA), and PG (specific RIA). Uterine contractility was recorded as the hourly area under the contraction curve and expressed as HCA in mmHg·sec/hr. Increases in the HCA occurred at 28 hrs (14-36) after inoculation in all 4 monkeys and led to progressive cervical dilatation in 3 of 4. These contractions were of high-amplitude, low-frequency, and long-duration. AF TNF rose from 48 pg/ml before inoc. to 20,000 pg/ml 9 hrs (6-14) after inoc. and 20 hrs (8-34) before increases in the HCA. Parallel increases in IL-1B (from 10 pg/ml to 1,142 pg/ml), PGE_2, and $PGF_{2\alpha}$ occurred 18 hrs (12-24) after inoc. and 10 hrs (2-18) prior to increases in the HCA. In contrast, spontaneous term labor in 4 control monkeys was not associated with increases in AF TNF or IL-1B and contractions were of high-amplitude, high-frequency, and short-duration. We conclude: 1) AFI with GBS leads to a predictable increase in uterine contractility which is different than spontaneous labor; and 2) in AFI, increases in AF cytokines and PG occur prior to increases in uterine contractility.

38 DOES THE RISK OF PERINATAL TRANSMISSION OF HIV-1 INCREASE WITH SUBSEQUENT PREGNANCIES? RR Viscarello, NJ DeGennaro*, YG Gollin*, WA Andiman*, JC Hobbins, Yale University School of Medicine, New Haven, CT.

Early reports of the rate of vertical transmission of HIV-1 in mothers who had delivered an index child with AIDS or ARC were as high as 65 to 80%. Recent, prospective studies have estimated the rate to be 7 to 33%. No study to date has examined the transmission rate in successive pregnancies. We studied 62 infants born to 27 HIV-positive women to determine if the risk of perinatal transmission of HIV-1 increases with subsequent pregnancies. There were 23 Blacks, three Hispanics, and one Caucasian. Eighteen of the women were current or previous IVDAs and 9 were infected heterosexually. Nineteen women had two pregnancies while infected and 8 had three. There are 14 infants currently CDC Stage P0 who were excluded from the analysis. The remaining 48 infants were classified according to CDC criteria into three groups: Seroreverted, P1, and P2, and stratified according to birth order. No statistically significant difference was found between infant disease status and birth order using Chi-square analysis. Mean gestational age at time of delivery and mean birth weight were inversely-related to birth order as follows: Pregnancy # 1: 37.3 wks ± 3 and 2814g ± 532; Pregnancy # 2: 36.6 wks ± 3 and 2768g ± 427; Pregnancy # 3: 35.3 wks ± 2 and 2226g ± 795. Maternal factors, including low CD4 cell count, positive HIV p24 antigen status, and maternal CDC Group IV disease (AIDS), were positively correlated with the presence of disease in the infant, but not with birth order. Our data does not support an increased risk of vertical transmission of HIV-1 with successive pregnancies. Markers of maternal viremia or immunodeficiency may be more accurate predictors of transmission of HIV-1. (This research was partially supported by a grant from the American Foundation for AIDS Research and the Pediatric AIDS Foundation AmFAR/PAF #S0034-7).

39 HYPOXIC ACIDEMIA DECREASES LEFT VENTRICULAR END-SYSTOLIC ELASTANCE IN FETAL SHEEP. R.M. Lewinsky[X], R.S. Szwarc[X], L.N. Benson[X], J.W.K. Ritchie. University of Toronto, Toronto, Ontario, Canada

End-systolic elastance, the slope of the end-systolic pressure volume relationship, is a relatively load and heart rate insensitive measure of the intrinsic contractile properties of the myocardium. Using the conductance catheter technique, we measured the effects of hypoxic acidemia on left ventricular (LV) end-systolic elastance in anaesthetized, 133 day gestation, *in utero* fetal sheep (n=7). Conductance and Millar catheters were introduced into the LV through a carotid artery cutdown. Fetuses were rendered progressively hypoxic and acidemic by embolization of the fetal placenta with repeated injections of $5{\cdot}10^5$ 50μM plastic spheres. We recorded pressure-volume data and arterial pH at 15 minute intervals. End systolic elastance was computed using a single beat method of extrapolating maximum isovolumic pressure. A gradual drop in fetal pH from 7.32 ± 0.06 (mean ± SD) to 6.95 ± 0.04 caused a significant decrease in LV elastance. A linear relationship was found to exist between elastance and pH, correlation coefficients ranging from 0.85 to 0.98, with a mean decrease of 4.42 mmHg/ml per 0.1 unit drop in pH. This decrease in elastance was gradual and extended over the entire clinically important range of pH, rather than being a terminal event. This study which, to our knowledge, is the first to use the conductance catheter to measure fetal LV function, shows that hypoxia acidemia adversely affects myocardial contractility, and suggests that indices of myocardial function should be explored as a means of improving upon the diagnostic accuracy provided by current monitoring methods.

40 DO ABNORMAL STARLING'S FORCES CAUSE FETAL HYDROPS IN RED CELL ALLOIMMUNIZATION?

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The etiology of fetal hydrops in anemic fetuses secondary to maternal red cell alloimmunization remains undefined. To investigate this problem, we studied 30 fetuses undergoing 56 intravascular transfusions (IVT). At 41 procedures, no ultrasound evidence of hydrops was noted (gestational age: mean +/- SD: 28.5 +/- 3.8 wks), while hydrops was present at 15 IVT's (25.9 +/- 4.1 wks). **Methods:** At IVT, umbilical venous (UVP) and amniotic fluid pressures (AP) were measured using a neonatal blood pressure monitor. Corrected UVP was determined by subtracting the AP from the absolute UVP. An aliquot of fetal blood was obtained and serum colloid osmotic pressure (COP) determined. Unpaired t tests were used for comparison; $p < 0.05$ was considered significant. **Results:** Hydropic fetuses had lower hematocrits than non-hydropic fetuses (17.7 +/- 7.3% vs 29.4 +/- 6.8%; $p < 0.001$). The COP-UVP gradient was lower in hydropic fetuses (0.9 +/- 4.1 vs 3.5 +/- 3.8 mm HG; $p = 0.03$). When the components were analyzed separately, UVP was not different between the two groups (8.0 +/- 3.8 vs 7.5 +/- 4.0 mm HG; p = NS); however COP was significantly lower in fetuses with hydrops (8.9 +/- 2.8 vs 11.0 +/- 2.1 mm HG; $p < 0.01$). **Conclusions:** Portal hypertension as reflected by UVP is unaltered in the anemic fetus with hydrops. Hypoproteinemia as reflected by COP may contribute to the pathophysiology of immune hydrops.

41 LONGITUDINAL CHANGES IN BASAL HEPATIC GLUCOSE PRODUCTION AND SUPPRESSION DURING INSULIN INFUSION IN NORMAL PREGNANT WOMEN

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The purpose of this study was to prospectively evaluate basal hepatic glucose production and suppression during insulin infusion longitudinally in pregnancy. Six multigravid, non-obese women were studied (mean±SD) 2.2±.3 months prior to conception (P) and again at 12-14 (E) and 34-36 (L) weeks gestation. Body composition was estimated by underwater weighing with correction for residual lung volume. Basal hepatic glucose production was estimated using a prime/constant infusion of 6-6 dideuterated (D2) glucose for 3 hours. Suppression of hepatic glucose production was estimated during insulin infusion with the hyperinsulinemic-euglycemic clamp at an insulin infusion of 40 mU/m2. There was a significant ($p=.005$) 30% increase in hepatic glucose production with advancing gestation (P-127.3±23.8, E-122.9±28.2, L-166.2±20.2 mg/min). This increase in hepatic glucose production remained significant ($p=0.05$) when corrected for fat free mass (P-2.74±0.23, E-2.62±0.35, L-3.14±0.36) and despite a significant ($p=0.02$) increase in fasting insulin (P-6.3±3.2, E-5.0±2.0, L-10.4±3.8 uU/ml). However, during insulin infusion hepatic glucose production was almost completely suppressed i.e. >90% throughout gestation. In summary, basal hepatic glucose production significantly increases to meet fetal/placental carbohydrate needs by late gestation but remains sensitive to infusion of insulin throughout gestation. Supported by NIH22965-01.

42 ENDOTHELIUM-DERIVED RELAXING FACTOR MEDIATES ESTROGEN-INDUCED INCREASES IN UTERINE BLOOD FLOW.

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Administration of estrogen results in significant increases in uterine blood flow (UBF) which may be mediated by endothelium-derived relaxing factor (EDRF). Eleven nonpregnant oophorectomized ewes were given 1 μg/kg of estradiol-17β and at the peak of the estrogen response received bolus injections of the EDRF inhibitor L-nitroarginine methyl ester (L-NAME). Estradiol-17β administration increased UBF from 19 ± 7 to 153 ± 35 ml/min. Local uterine artery administration of L-NAME (1-30 mg) led to dose-related decreases in UBF (26-63%) without any change in blood pressure (BP), heart rate (HR), or cardiac output (CO). In a second study, systemic administration of L-NAME (1 to 30 mg/kg) led to significant dose-related increases in BP (26% max), and decreases in HR (34% max), and CO (42% max). UBF in this group increased from a baseline of 4±3 ml/min to a maximum of 126±19 ml/min. Systemic administration of L-NAME decreased UBF in a dose-dependent fashion reaching a maximum at 30 mg/kg of 81±4%. Subsequent administration of the EDRF precursor, L-arginine (100 mg/kg), partially reversed the inhibition of L-NAME on BP, HR, CO and increased UBF from 25±4 to 65±16 ml/min. Conclusion: Estrogen produces uterine vasodilation which is antagonized by L-NAME, an EDRF inhibitor, and reversed by L-arginine, an EDRF precursor.

43 **TUMOR NECROSIS FACTOR ALPHA (TNF-α) IN SECOND-TRIMESTER AMNIOTIC FLUID IS ASSOCIATED WITH IMPAIRED INTRAUTERINE FETAL GROWTH.** K. Heyborne[1], J. McGregor[1], S. Witkin[2x], G. Henry[3x], Departments of Ob/Gyn, University of Colorado[1] and Cornell Medical Center[2] and Reproductive Genetics[3], Denver, CO

Inadequate intrauterine fetal growth is a common cause of low birth weight and perinatal morbidity. To investigate whether abnormal immune system activation is involved in the pathogenesis of some instances of intrauterine growth retardation, TNF-α/cachexin, a cytokine linked to impaired cell growth, was measured in amniotic fluid. Bioactive TNF-α was measured using a sensitive and specific WEHI cell assay. Amniotic fluid samples were obtained by genetic amniocentesis at 14 - 20 weeks gestation. In a case-control study, samples obtained from 25 gestations that resulted in small for gestational age infants (SGA, birth weight less than 10%) were compared with 35 samples obtained from gestations resulting in the birth of a term AGA infant. The two groups were not significantly different with regard to maternal age, race, socioeconomic status, or gestational age at amniocentesis. All infants had normal karyotypes, and gestational ages were confirmed by ultrasound at the time of amniocentesis. Groups were compared using the Wilcoxon rank sum test. Elevated amniotic fluid TNF-α was associated with SGA birth, $P = 0.014$. Using a threshold of 11 pg/ml, the assay had a sensitivity of 48% for the detection of SGA birth, with a specificity of 83%. TNF-α noted in amniotic fluid at second trimester amniocentesis is associated with impaired intrauterine growth. Abnormal immune system activation as manifest by increased TNF-α may mediate impaired fetal growth in some cases.

44 **COCAINE DIRECTLY AFFECTS SIGNAL TRANSDUCTION IN HUMAN MYOMETRIAL CELLS.** F. Hertelendy[X], M. Molnár[X], Dept. Ob/Gyn, St. Louis University Med. Ctr., St. Louis, MO

Cocaine has been reported to stimulate myometrial activity both in vivo and in vitro. The aim of this study was to test the hypothesis that cocaine directly interferes with adenylate cyclase-mediated signal transduction, thereby attenuating responses to agonists that raise intracellular cAMP, known to promote uterine relaxation. Exposure of human myometrial cells (HMC) to cocaine for up to 4 days dose-dependently inhibited (max. 70%) isoproterenol (ISO)-, $PGF_{2\alpha}$- and forskolin (FSK)-stimulated cAMP production. Similarly, in HMC permeabilized with α-toxin, cocaine (0.01-10μM) attenuated ISO-, FSK- and GTPγS-promoted cAMP synthesis. However, whereas in intact cells the inhibitory effect was correlated with the duration of cocaine exposure (1-4 days), in the permeabilized system it was immediate. Pretreatment of HMC with pertussis toxin prevented the inhibitory action of cocaine, suggesting a postreceptor site at which cocaine interferes with signal transduction. It is concluded that at least one of the mechanisms by which cocaine promotes uterine contractility is the attenuation of cAMP generation by uterine relaxants.

Poster Session I

Thursday, February 6, 1992
10:30 a.m.-12:30 p.m.

Grand Salons I-IV

CATEGORIES	POSTER NOS.
Hypertensive Disease of Pregnancy	45-77
Medical Complications of Pregnancy	78-132
Intrapartum Fetal Evaluation	133-144

45 **Withdrawn at authors' request.**

46 **MID-GESTATIONAL HYPERINSULINEMIA AND DEVELOPMENT OF PREECLAMPSIA.** JR Sowers[x], AA Saleh T Niyogi[x], P Standley[x], SF Bottoms, GS Norman[x], MB Zemel[x], PC Zemel[x], BE Johnson[x], RJ Sokol, Department of Medicine, Ob/Gyn, Wayne State University School of Medicine, Detroit, MI and Department of Nutrition, University of Tennessee, Knoxville, TN

There is evidence that hyperinsulinemia/insulin resistance play a role in development of hypertension. Accordingly, in our ongoing longitudinal study of pregnancy-induced hypertension we have measured fasting levels of insulin and glucose at 20 to 21 weeks of gestation in 140 nulliparous black women followed prospectively to delivery and with complete data. To test the hypothesis that hyperinsulinemia may be related to development of preeclampsia discriminant analysis of mean arterial pressure (MAP), fasting plasma insulin levels and left lateral forearm vascular resistance were examined as predictors of preeclampsia with control of two factors known to be related to insulin levels, gestational age and pre-gestational body mass index. Characteristics of eleven gestational hypertensives were not different from that of normals and thus they were placed in the control group. Women who developed preeclampsia had fasting plasma insulin levels at 21 weeks of 51.2 ± 47(S.D.) and controls at 20 weeks had values of 29.2 ± 31.5 μU/ml. Only MAP ($F(4,135)=8.8$, $p<0.01$) and insulin ($F1,135)=6.5$, $p<0.05$) were related to development of preeclampsia ($F(4,135)=4.39$, $R^2=11.5\%$). The finding that mid-gestational insulin levels characterize the subsequent development of preeclampsia with control for increased MAP supports the hypothesis that hyperinsulinemia/insulin resistance may contribute to the pathogenesis of preeclampsia.

47 **EFFECT OF PROGESTERONE RECEPTOR BLOCKADE ON RENIN SYNTHESIS AND SECRETION FROM ENDOMETRIAL STROMAL CELLS: PULSE EXPERIMENT.** D. Shah, R. Riehl,[x] Dept. of OB/GYN, The University of Texas Health Science Center, San Antonio, TX.

The etiology of preeclampsia is largely unknown, but a decrease of uteroplacental blood flow is an important aspect of pathophysiology. Uterine renin may have a role in regulation of uterine blood flow. We have recently shown, by RIA for angiotensin I, that the endometrial stromal cell is the specific cell responsible for uterine renin secretion. Furthermore, progesterone increases this renin secretion. We examined new renin synthesis and secretion by pulse experiment to study the effect of progesterone receptor blockade. Endometrial specimens were obtained from benign uteri removed at hysterectomy for clinical indications. Isolation and culture of a highly purified population of stromal cells were established by a previously described method. After initial 2-day incubation in serum-containing media, the cells were maintained in culture up to 9 days in serum-free DME/F-12 nutrient mixture with ITS supplement. Four experimental groups [1. No steroid control (C), 2. Progesterone alone (P), 3. RU-486 alone (R), and 4. progesterone + RU 486 (PR)] were established from day 3 onwards. Culture media were changed at 2-day intervals. On the day of the experiment, after 6 hours of treatment with fresh culture fluid, all culture fluids were removed and replaced by methionine-free DME/F-12 + ITS mixture containing ^{35}S-labeled methionine. After 16 hours of endogenous radiolabeling, culture fluids were collected the next day and proteins were TCA precipitated. SDS-polyacrylamide gel electrophoresis was carried out on secretory proteins. Results: Secretory proteins show a 55 KD band representing prorenin and a 44KD band representing renin. The renin band was faint in the control group. The progesterone treatment group primarily showed renin and the prorenin band was essentially absent. Treatment with receptor blockade alone or with progesterone had a pattern similar to that of the control group. This suggests that progesterone treatment of stromal cells (*in-vitro* decidualization) induces secretion of renin in preference to prorenin and that receptor blockade effectively blocks the preferential renin secretion.

48 SEVERE FETAL IUGR MAY ANTEDATE CLINICAL EVIDENCE OF PREECLAMPSIA BY SEVERAL WEEKS. A. Nova,[x] B. Sibai, J. Barton,[x] A. Khoury,[x] N. Meyer,[x] B. Mercer.[x] University of Tennessee, Memphis.

The association of preeclampsia with fetal intrauterine growth retardation (IUGR) is well known. We hypothesized that severe fetal IUGR may be an early finding for impending preeclampsia. The purpose of this study is to report the clinical, laboratory findings, and perinatal outcome in 43 normotensive women with well-established IUGR who ultimately developed preeclampsia. Materials and Methods: Inclusion criteria were: good dating criteria by dates, first visit and/or early USG, absence of chronic hypertension, documented IUGR by USG, singleton pregnancy, no hypertension and/or proteinuria at initial diagnosis of IUGR. Results: All 43 patients developed preeclampsia: 20 mild and 23 severe (5 with HELLP syndrome). The mean uric acid in these women was 6.6±1.4 mg/dl, and 40 (93%) had significant proteinuria. The mean interval from diagnosis of IUGR to onset of preeclampsia was 2.1 weeks (range, 0.43 to 6). Table summarizes clinical findings in these patients. Twenty-seven (63%) of the infants were <5th percentile and 16 (37%) were <10th percentile. There were 3 stillbirths and 11 neonatal deaths for a perinatal death rate of 32.6%. Thirty-one infants required admission to special care nursery for an average stay of 39 days (range, 4-203 days). Conclusions: IUGR may antedate impending preeclampsia. Thus, preeclampsia should be considered in differential diagnosis of normotensive patients presenting with severe IUGR. Such pregnancies are at high risk for severe preeclampsia, HELLP syndrome, preterm delivery, and poor perinatal outcome.

	Means±SD	Range
SBP at diagnosis of IUGR (mmHg)	120±9.8	102-138
DBP at diagnosis of IUGR (mmHg)	75±9.5	60-86
GA at diagnosis of IUGR (wk)	29.4±4.2	22-37
GA at diagnosis of preeclampsia (wk)	31.3±3.9	25-39
Delivery ga (wk)	31.7±3.8	25.6-39
SBP at onset of preeclampsia (mmHg)	156±21	130-220
DBP at onset of preeclampsia (mmHg)	101±10	86-110

SBP=systolic blood pressure, DBP=diastolic blood pressure

49 UMBILICAL VENOUS PLASMA ENDOTHELIN IS NOT INCREASED IN PREECLAMPSIA. A.Nova,[X] J. Barton,[X] M.D. Mitchell, B.M. Mercer, [X]B.M. Sibai, University of Tennessee, Memphis, and University of Utah, Salt Lake City.

Plasma endothelin (ET) levels are usually increased in conditions characterized by endothelial damage and local tissue hypoxia. We previously reported that women with preeclampsia, particularly those with HELLP syndrome, have significantly higher endothelin levels than women with normotensive pregnancies. The purpose of this investigation is to compare umbilical vein plasma ET levels in preeclampsia and normotensive pregnancies. Methods: The study population included 7 women with preeclampsia and 12 with normotensive pregnancies. All but one woman delivered vaginally at term. Samples were collected immediately after cord clamping using cold vacutainer tubes containing EDTA and aprotinin. The blood was immediately cold centrifuged and plasma fraction was then stored at -70°c. Plasma was analyzed for endothelin using an RIA technique (Amersham Corp.). Results: As expected, preeclamptic women had significantly higher systolic blood pressures (152 ± 13 v 118 ± 10 mmHg, $p < 0.0001$) and higher diastolic pressures (97 ± 9.4 v 69 ± 9.2, $p < 0.0001$). There were no differences between the two groups regarding either gestational age at time of delivery or cord vein ET levels (Table). However, cord blood ET levels are significantly higher than previous reported maternal levels (preeclamptics 12.87 ± 3.6 v 5.5 ± 0.3, $p < 0.001$ and normotensive 12.2 ± 2.7 v 3.8 ± 0.3, $p < 0.0001$). Conclusions: Fetal secretion of ET is not increased in preeclampsia. The increase in umbilical ET at delivery suggests a role for ET in perinatal circulatory adaptation.

	Preeclampsia n = 7	Normotensive n = 12
Gestational age (wk)	37.7 2.6	39.0 1.5
Umbilical vein ET (fmole/ml)	12.87 3.62	12.21 2.69

50 **LOW-DOSE ASPIRIN (ASA) INHIBITS LIPID PEROXIDES (LPO) AND THROMBOXANE (TX), BUT NOT PROSTACYCLIN (PGI), IN PREGNANT WOMEN.** SW Walsh,[X] Y Wang,[X] HH Kay, MC McCoy.[X] Depts OB/GYN, Medical College of Virginia, Richmond, VA and Duke Univ., Durham, NC

Preeclampsia is associated with an imbalance of increased TX and decreased PGI, and recently reported an abnormal increase of LPO (AJOG, Dec., 1991). LPO are toxic compounds that damage cells and inhibit PGI synthesis. Low-dose ASA therapy reduces the incidence of preeclampsia, presumably by selective inhibition of TX to restore a balance between TX and PGI. However, the effectiveness of low-dose ASA might also relate to inhibition of LPO. To test this, 10 women at risk of preeclampsia were placed on low-dose ASA therapy (81 mg/day) between 19-33 wks of gestation. Plasma samples were collected before ASA and after 3-4 days and 3-4 wks of ASA. Samples were analyzed for TX and PGI by RIA of their stable metabolites, TXB_2 and 6-keto PGF_{1a}, and for LPO by H_2O_2 equivalents. Low-dose ASA significantly decreased ($P<0.05$) both LPO (130 ± 18 vs 92 ± 11 and 68 ± 9 nmol/ml, mean ± SE) and TX (502 ± 67 vs. 138 ± 67 and 8 ± 5 pg/ml), but it did not affect PGI (55 ± 10 vs 41 ± 8 and 40 ± 11 pg/ml). Conclusion: Low-dose ASA selectively inhibits both LPO and TX without affecting PGI. Speculation: This selective inhibitory action of low-dose ASA may account for its effectiveness in the prevention of preeclampsia. HD 20973.

51 INSULIN CONCENTRATIONS IN CHRONIC HYPERTENSIVE PREGNANT WOMEN. E. Rey,[X] A. Bonin,[X] Dept. Ob/Gyn, Sainte-Justine Hospital, Montreal (Quebec), Canada.

In order to investigate the state of insulin resistance of chronic hypertensive pregnant women, we studied glucose and insulin responses to oral glucose loads in these patients. Chronic hypertension was defined as essential hypertension known before pregnancy. Gestational diabetic and treated chronic hypertensive women were excluded. Serum capillary glucose was determined by the glucose oxidase method and insulin levels by radio-immunoassay. One hundred and forty-seven (147) euglycemic lean normotensive pregnant women (Body mass index (BMI) = 21.8 ± 0.2), 30 euglycemic obese normotensive pregnant women (BMI = 32.5 ± 1.0) and 25 euglycemic lean chronic hypertensive pregnant women (BMI = 23.7 ± 0.6) received a 50 gm oral glucose load. Insulin levels and insulin to glucose ratio at one hour were significantly higher in normotensive obese and hypertensive women than in normotensive lean women ($p < 0.001$ and $p < 0.01$). Following a 100 gm glucose load, glucose and insulin concentrations were measured for a three-hour period in 26 euglycemic lean normotensive women (BMI = 21.9 ± 0.5), 27 euglycemic lean hypertensive women (BMI = 23.5 ± 0.6) and 57 lean gestational diabetic women (BMI = 23.2 ± 0.5). Hypertensive and gestational diabetic women disclosed significantly higher insulin concentration and insulin to glucose ratio than normotensive women at 2 and 3 hours ($p < 0.05$). These data suggest that mild hypertensive pregnant women, as obese and gestational diabetic patients, display a state of insulin resistance.

52 DOPPLER EVIDENCE OF RENAL HYPERPERFUSION IN PREECLAMPSIA. JF Smith, GJ Gilson, GO Del Valle, G Joffe, LA Izquierdo, M Chatterjee, LB Curet. University of New Mexico, Albuquerque, NM

To further clarify the renal hemodynamic changes associated with preeclampsia, we initiated a prospective study of maternal renal arcuate artery Doppler velocimetry changes associated with that disease. Twelve preeclamptics were compared to 21 normotensive patients. All preeclamptics had blood pressures of at least 140/90, and proteinuria of at least 300 mgs/24 hours. Renal arcuate arteries were identified at the bases of the renal pyramids in a subcostal, transverse view; for consistency, the right kidney was chosen for insonation. A sample volume of 3-5 mm and wall filter setting of 50 Hz was used. FINDINGS: Compared to the normotensive patients, the preeclamptics had a significantly lower mean pulsatility index (0.89 vs 1.18, P=0.01) of the maternal renal arcuate artery. We have found that the increase in pulsatility index in the late third trimester that occurs in normotensive pregnancies may not occur in preeclamptics; confirmatory longitudinal studies are necessary. The data presented here suggests increased renal blood flow in preeclampsia and is consistent with the renal hyperperfusion model for this disease.

53 ERYTHROCYTE MEMBRANE FLUIDITY IN PATIENTS WITH PREECLAMPSIA.

L. Sanchez-Ramos MD, M. Jordan MD[X], D L Mollitt MD[X], D Adair MD[X], M. T. Cullen, M.D., Divisions of Maternal-Fetal Medicine and Pediatric Surgery, University of Florida, Jacksonville, FL.

Recent information indicates that membrane lipids and other cell components are disrupted in patients with preeclampsia. Under physiologic conditions, the lipids of biologic membranes are in liquid-crystalline state. Although the hydrophobic interactions and the hydrogen bonding that occur between lipids maintain the integrity of the lipid bilayer, they also permit appreciable amounts of molecular motion within that bilayer. The process of molecular motion within the membrane is referred to as fluidity. Fluorescence studies of membrane fluidity were conducted using washed erythrocytes prepared from 9 patients with severe preeclampsia, including 4 with the HELLP syndrome, and an equal number of normotensive controls. The fluidity of the hydrocarbon and liquid-aqueous interface regions of cell membranes was determined at 37°C by fluorescence spectroscopy using the lipid probe 1-[4-(trimethylamino)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH). The rotation rate of TMA-DPH in labeled red cell membranes did not differ between preeclamptics and normotensive controls (0.286±.02 and 0.280±.02). However, the rotation rate in patients with HELLP was less than normotensive controls (0.307±.01 and 0.280±.02; p<.001) suggesting decreased membrane fluidity. Such changes may be associated with alterations of the red cell membrane leading to hemolysis.

54 THE EFFECT OF MAGNESIUM SULFATE ON MATERNAL BRAIN BLOOD FLOW IN MILD PREECLAMPSIA: A RANDOMIZED PLACEBO-CONTROLLED STUDY

Michael A. Belfort,[X] Kenneth J. Moise, Jr., George Saade.[X] Dept. Ob/Gyn, Baylor College of Medicine, Houston, Texas.

INTRODUCTION: The effects of magnesium sulfate on maternal cerebral blood flow (CBF) in PIH are not well described. We therefore studied the acute effect of a 6 gram dose of magnesium sulfate on CBF in mild PIH. METHODS AND MATERIALS: Twelve patients were randomized to 2 groups and studied with Doppler ultrasound before and after infusion of either a 6 gram intravenous loading dose of magnesium sulfate, or placebo. The change in the pulsatility index (Delta-PI) in the middle cerebral (MCA), common carotid (CCA), and internal carotid (ICA) arteries was compared (unpaired student t-test). RESULTS: All data* is presented as the change in the pulsatility index (Delta-PI).

	Placebo Group (n=6)	Magnesium Sulfate Group (n=6)	p
MCA	-0.02 +/- 0.06	-0.16 +/- 0.09	0.01
CCA	-0.05 +/- 0.27	-0.28 +/- 0.35	NS
ICA	-0.01 +/- 0.29	-0.14 +/- 0.24	NS

* = Mean +/- SD

Magnesium sulfate significantly reduced the pulsatility index in the MCA, without significantly changing the indices of the carotid vessels. CONCLUSIONS: Magnesium sulfate vasodilates the small intracranial vessels distal to the MCA, and may help to prevent seizures by relieving cerebral ischemia rather than by suppressing neuronal activity.

55 THE EFFECT OF MAGNESIUM SULFATE ON MATERNAL AND FETAL BLOOD FLOW IN PREGNANCY-INDUCED HYPERTENSION (PIH).

Michael A. Belfort,[X] Kenneth J. Moise, Jr., George Saade.[X] Dept. Ob/Gyn, Baylor College of Medicine, Houston, Texas.

The acute effects of magnesium sulfate (MgSO4) on maternal and fetal cerebral blood flow are not well described in PIH. MATERIALS AND METHODS: Twelve patients with PIH were prospectively studied with transcranial and transabdominal color Doppler, before and after infusion of a 6 gram IV dose of MgSO4. The maternal vessels studied included the middle cerebral (MCA), common carotid (CCA), and internal carotid (ICA) arteries. The fetal vessels included the middle cerebral, renal, and umbilical arteries. The maternal circulation in the placental base plate was also imaged. RESULTS: Doppler data (mean +/- SD; all values are pulsatility index unless otherwise indicated. * = S/D ratio.

MATERNAL VESSELS	n	Baseline	After MgSO4	p
MCA	12	0.77 +/- 0.07	0.69 +/- 0.1	>0.02
CCA	12	2.34 +/- 1.0	1.98 +/- 0.5	NS
ICA	12	1.23 +/- 0.6	1.11 +/- 0.3	NS
FETAL VESSELS				
MCA	9	1.53 +/- 0.2	1.61 +/- 0.3	NS
Renal Artery	7	2.17 +/- 0.3	2.23 +/- 0.4	NS
Umbilical Artery*	10	2.86 +/- 0.9	2.40 +/- 0.4	NS
PLACENTAL VESSELS				
Maternal Placental*	8	1.72 +/- 0.2	1.70 +/- 0.3	NS

CONCLUSIONS: A 6 gram bolus dose of MgSO4 significantly vasodilated vessels distal to the maternal MCA, without significantly affecting the other maternal or fetal vessels studied.

56 THE EFFECT OF MAGNESIUM SULFATE ON BLOOD FLOW IN THE MATERNAL RETINA IN MILD PREGNANCY-INDUCED HYPERTENSION (PIH): A PRELIMINARY STUDY.

Michael Belfort.[X] Dept. Ob/Gyn, Baylor College of Medicine, Houston, Texas.

There are few data on retinal blood flow (RBF) in patients with PIH. The central retinal artery (CRA) is an end branch of the internal carotid artery (ICA) and may reflect changes in cerebral blood flow (CBF). This prospective study was designed to assess the effects of a bolus infusion of 6 grams of MgSO4 on the CRA in patients with PIH. MATERIALS AND METHODS: Five women with PIH undergoing induction of labor underwent color flow Doppler ultrasound examination of the CRA before and after IV MgSO4 (6 grams in 100 ml 5% dextrose water over 20 minutes). BP, HR, Pulsatility Index (PI), Resistance Index (RI), angle-corrected flow-velocity were recorded. RESULTS: The central retinal artery was easily and reliably visualized. Six grams of MgSO4 significantly reduced (p<0.03) the PI from 0.93 (SD 0.16) to 0.76 (SD 0.19) and RI from 0.57 (SD 0.05) to 0.47 (SD 0.08). Flow-velocity increased post MgSO4. CONCLUSIONS: MgSO4 acutely vasodilates small arteries in the retina, increasing blood velocity: similar changes may occur in other branches of the ICA supplying the brain. This technique may allow better monitoring of the effects of therapeutic interventions on CBF in patients with PIH.

57 **EUTHYROID SICK SYNDROME IN PRE-ECLAMPSIA.** T. T. LAO[X], R. K. H. CHIN[X], N. S. PANESAR[X], R. SWAMINATHAN[X]. Depts Ob/Gyn and Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong.

The Euthyroid Sick Syndrome (low plasma levels of thyroid hormones in euthyroid patients) is often found in severely ill non-pregnant patients. To examine the occurrence of this Syndrome in pre-eclampsia, 53 consecutively admitted proteinuric pre-eclamptic patients (ACOG criteria), who had no history of thyroid disease and not requiring urgent delivery for maternal/fetal reasons, were studied together with 30 normotensive third trimester controls. Blood was collected in a heparinized tube and the plasma separated by centrifuge at 4°C and stored until assayed with Syva EMIT for total thyroxine (TT4) and RIA (Diagnostic Products Corporation) for free thyroxine (FT4), total and free tri-iodothyronine (TT3 and FT3), and thyrotropin (TSH). All the pre-eclamptic patients had thyroid hormone levels within the normal non-pregnant range. Compared to controls, 24 (45.3%) pre-eclamptic patients had decreased levels in one or more thyroid hormones in the form of (1)low TT4 and TT3 ± low FT4 and/or FT3, n=7; (2)low TT4 ± low FT4 and/or FT3, n=10; (3)low TT3 ± low FT4 and/or FT3, n=2; (4)low FT4 and/or FT3 only, n=5. Elevated TSH was found in 8 (33.3%) of these patients and 6 (20.7%) of the other pre-eclamptic patients. There was no consistent trend in the pattern of abnormal thyroid hormone levels. There was no difference in the incidence of intrauterine growth retardation in pre-eclamptic patients with abnormal (45.8%) and normal (34.5%) thyroid hormone levels. In conclusion, the Euthyroid Sick Syndrome is a frequent occurrence and probably a reflection of the severity of the maternal condition in pre-eclampsia.

58 **MAGNESIUM SULPHATE (MGSO4) THERAPY IN PREECLAMPSIA IS ASSOCIATED WITH INCREASED URINARY CYCLIC GUANOSINE MONOPHOSPHATE (CGMP) EXCRETION.** JR Barton[X], BM Sibai, R. Ahokas[X], WD Whybrew[X], BM Mercer[X]. University of TN, Memphis.

Endothelium-derived relaxing factor (EDRF) is a mediator of vascular smooth muscle relaxation. Assays for EDRF are difficult due to its short half life. However, it has been found to stimulate the formation of cGMP which is more stable. Since both normotensive and hypertensive pregnancies are associated with significant changes in vascular smooth muscle tone, we investigated maternal urinary cGMP levels as a potential indicator of EDRF activity. **Methods:** 57 women with preeclampsia (38 prior to and 19 during $MgSO_4$ infusion) and 14 normotensives were studied. 24hr urine samples were collected in the third trimester and an aliquot was stored at -70°C. Urinary cGMP was analyzed using a RIA technique (Amersham Corp.) and expressed as pmole per mg of urinary creatinine to standardize for renal function. **Results:** There was no difference in gestational age at time of urine collection between the two groups. Urinary cGMP levels expressed as mean ± SD were similar between the two groups (table). Although not statistically different, preeclamptic women not receiving $MgSO_4$ had lower cGMP levels than normotensives. There was no correlation between cGMP levels and severity of hypertension. Preeclamptic women receiving $MgSO_4$ had significantly higher levels of cGMP ($p=0.02$) than those not receiving $MgSO_4$. **Conclusions:** These preliminary results indicate that cGMP excretion increases in patients with preeclampsia during $MgSO_4$ infusion. This may indicate that the vascular smooth muscle relaxation effects of $MgSO_4$ are mediated by EDRF as determined by increased cGMP production.

		GA (wks)	cGMP (pmole/mg cr)	
Normotensive	(n=14)[+]	32.9±3.3	751±498	
Preeclampsia	(n=57)	34.1±3.8	632±363	[+]p=0.12
without MgSO4	(n=38)[*+]	34.6±3.5	555±344	[*]p=0.02
with MgSO4	(n=19)[*]	33.4±4.0	786±360	

59 **URINARY ENDOTHELIN-1: NOT A USEFUL MARKER FOR PREECLAMPSIA.** JR Barton,[X] BM Sibai, WD Whybrew[X], BM Mercer[X]. University of Tennessee, Memphis.

Endothelin-1 (ET-1), a 21-residue peptide produced by endothelial cells, is one of the most potent vasoconstrictors identified in humans. ET-1 serum levels are reportedly increased in situations of endothelial damage, including preeclampsia. We investigated maternal urinary ET-1 levels to determine if a similar increase could be detected and used as a marker for preeclampsia. **Methods:** 50 women with preeclampsia (36 prior to and 14 during $MgSO_4$ infusion) and 11 normotensive gravidas were studied. 24 hour urine samples were collected in the third trimester and an aliquot was stored at -70°C. Urinary ET-1 was analyzed using an RIA technique (Amersham Corp.) and expressed as femtomoles ET-1 per mg of urinary creatinine to standardize for renal function. **Results:** There was no difference in gestational age at time of urine collection between the two groups. Urinary ET-1 levels expressed as mean ± SEM were similar between the two groups (table). There was no correlation between ET-1 levels and severity of hypertension. Preeclamptic women receiving $MgSO_4$ had similar levels of urinary ET-1 to those not receiving $MgSO_4$.

		GA (wks)	ET-1 (fmole/mg cr)
Normotensive	(n=11)	32.4 ± 0.7	62.7 ± 7.5
Preeclampsia	(n=50)	33.4 ± 0.6	79.8 ± 9.3
without MgSO4	(n=36)	34.1 ± 0.7	78.7 ± 9.3
with MgSO4	(n=14)	32.2 ± 0.9	82.7 ± 23.9

Conclusions: These results indicate that ET-1 excretion is not significantly increased in patients with preeclampsia. Thus, urinary ET-1 excretion is not a good marker for preeclampsia.

60 **IS ACCELERATED FETAL LUNG MATURITY EQUATED WITH HYPERTENSIVE DISORDERS?** O. Langer, J. Piper,[X] A. Samueloff,[X] L. Ridgway, M. Berkus, E. Xenakis.[X] Department of OB/GYN, University of Texas Health Science Center at San Antonio, TX.

Increased fetal lung maturity for a given gestational age (GA) has consistently been associated with hypertensive disorders However, the definitive role of hypertensive disorders in pregnancy has not been established. In order to investigate this relationship, 6019 consecutive women with hypertensive disorders and 6358 non hypertensive consecutive preterm deliveries serving as controls were studied. Women with diabetes and twin gestations were. For purpose of analysis, patients were stratified by gestational week. The incidence of hyaline membrane disease in each group was:

GA	*CHTN (N=1807)*	*PET (N=4212)*	*CONTROL (N=6358)*
26 29	13.0*	29.4	29.6
30 32	9.8	17.4	14.4
33-35	8.6	4.0	4.6
36 37	1.2	1.0	1.6
38-HI	0.3	0.1	

*Significant difference $p < .01$ CHTN vs PET and CONTROL

The study further revealed: 1) comparison between and within each group revealed similarity in maternal age, infant gender, race and method of delivery; 2) at 30 weeks gestation, PET and control groups are at greater risk for developing RDS (RR 2.77, 95% CI 1.07-7.2) in comparison to the CHTN infants; 3) overall, a 2-fold higher risk for SGA was found in hypertensive disorders compared to controls. There was no significant difference in incidence of SGA between PET and CHTN subjects; and 4) CHTN subjects had significantly higher rates of stillbirth at each gestational age category in comparison to PET and control groups (overall odds ratio 3.9, 95% CI 2.8 5 4), respectively In conclusion, for the same gestational age group, PET and non hypertensive infants are at comparable risk for developing lung complications Conversely, the CHTN infant at 26 29 weeks gestation has a lower incidence of lung disease. This information will enhance the decision-making process when early delivery is considered in these high-risk fetuses.

61 **COCAINE: POSTPARTUM HYPERTENSION NOT A RISK WITH BROMOCRIPTINE USE.** YR Renfroe[X], RK Bhatia, SF Bottoms, DL Watson, and RJ Sokol. Wayne State Univ., Hutzel Hospital, Detroit, MI.

On the basis of two case reports of severe postpartum hypertension (PPH) and secondary cortical blindness, seizures, or pulmonary edema, it has been recommended that bromocriptine should not be used for lactation suppression in cocaine abusers. A postulated mechanism for cocaine to potentiate the dopaminergic effects of bromocriptine could be reuptake inhibition of both dopamine and norepinephrine at the presynaptic receptors. However, severe hypertension and its sequelae have also been reported with bromocriptine alone, especially in patients with preeclampsia. To investigate the relationship, if any, between bromocriptine, cocaine abuse, and postpartum hypertension, we studied 1065 patients, excluding those with antepartum hypertension. All patients had blood pressure determinations during 2 home visits by a nurse within 3 weeks postpartum. PPH (n = 178) was defined as persistent elevation $\geq$ 140/90 at either visit. Stepwise discriminant analysis with PPH as the dependent variable revealed that a history of preeclampsia ($p < 0.001$), obesity ($p < 0.01$), and parity ($p < 0.05$) were significant risk factors for PPH. Neither bromocriptine use (n = 773), cocaine abuse (n = 52), or their combination (n = 46) was significantly associated with PPH. None of the studied patients developed serious sequelae of PPH. We conclude that cocaine abuse is not a contraindication for the use of bromocriptine to suppress lactation.

62 TRANSCRANIAL ASSESSMENT OF MATERNAL CEREBRAL BLOOD FLOW VELOCITY IN NORMAL VS PREECLAMPTIC WOMEN; VARIATION WITH MATERNAL POSTURE

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Cerebral blood flow velocity (CBFV) can be extensively evaluated in adults using a 2 mHz transcranial doppler ultrasound. Middle Cerebral Artery (MCA) blood flow velocity is lower with the patient in the upright then in the supine position (Ultrashall Med 1986; 7: 248-52). We assessed MCA blood flow using a pulsed wave doppler with a 2 mHz. probe in five normotensive and five preeclamptic (PET) pregnant patients in both supine and standing positions to evaluate the effect of change in maternal position on CBFV. The MCA was insonated through a temporal ultrasound window at a depth of 5.2 cm. We assessed maximum systolic, minimum diastolic and mean MCA flow velocity. We found (1) PET women showed higher MCA velocity than normotensive women, (2) Normotensive women showed a fall in all aspects of MCA velocity, from supine to standing (5-12%), (3) PET women showed a significant rise ($P<.05$) (average 20% increase) in all aspects of MCA velocity from supine to standing.

		Max. Systolic (mm/sec)	Max. Diastolic (mm/sec)	Mean
Normo-	supine	87 +/- 15	31 +/- 6	50 +/- 8
tensive	standing	80 +/- 17	28 +/- 6	47 +/- 10
PET:	supine	80 +/- 17	39 +/- 12	55 +/- 15
	standing	94 +/- 14*	48 +/- 11**	66 +/- 13*

* P <.04, ** P <.01

Conclusions: These data show that maternal cerebral blood vessels react differently in pregnant PET women. The pathophysiology of this finding and whether clinically it can be used in a predictive fashion requires further investigation.

63 **ECLAMPSIA--TYPICAL FINDINGS WITH MRI.**

MA Dahmus[X]--JRBarton[X], BM Sibai, University of TN, Memphis.

Purpose: To study the MRI findings in patients with eclampsia, and to determine the clinical utility of this imaging modality. **Methods:** 25 patients with a diagnosis of eclampsia received cerebral magnetic resonance imaging. The scans were obtained from 0 to 18 days after the first seizure (median 6.6 days). The scan at 18 days was a rescheduled exam secondary to claustrophobia during the first study one week earlier. **Results:** Fourteen scans were normal (median of 5.5 days after the first seizure). One additional scan could not be completed due to claustrophobia, and was not rescheduled. The T2-weighted images were the most useful. Only 2 of the patients had abnormalities on the T1 weighted scans. No cerebral hemorrhage or atrophy was noted. Cortical edema in the posterior hemispheres (posterior parietal and occipital lobes) was the single most common finding--seen in all 11 abnormal scans. Infratentorial abnormalities were noted in two cases--edema in the midbrain and brain stem in 1 case, and cerebellar edema in the other case. Five patients had edema of the cortex and white matter. Three of the patients had focal neurologic deficits at admission, but one patient recovered before the time of scanning. The patient with normalization of neurologic function had only cortical edema on her scan (obtained at 18 days secondary to claustrophobia). The other 2 patients, who also had residual focal neurologic deficits at discharge, had both white matter edema and cortical edema, and one also had changes consistent with infarction. Three of the 7 patients who had scans more than one week after the first seizure activity had abnormal studies. These 3 patients had the most abnormal neurologic exams of all 26 patients. Patient management was not changed by MRI results, but the studies helped to exclude certain pathology (tumor, aneurysm, stroke) for which specific intervention might be useful. **Conclusion:** Cortical edema is the most common abnormality in eclamptic patients receiving cerebral MRI studies. We recommend limiting the use of MRI to patients with focal neurologic deficits, prolonged coma, or intractable seizures in order to exclude serious pathology for which specific therapy might be indicated.

64 **PROSTAGLANDIN PRODUCTION BY TROPHOBLAST OF NORMOTENSIVE AND PREECLAMPTIC PREGNANCIES**

M. Morgan, S. Silavin[X], D. Rice[X], Dept Ob/Gyn, Univ. of California Irvine, Orange, CA and Dept Ob/Gyn, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK

Preeclampsia, is hypothesized to have an alteration in placental production of thromboxane and prostacyclin. The purpose of this study was to determine if trophoblasts from preeclamptic (PIH) patients produce differential amounts of thromboxane and prostacyclin compared to normotensives (NORM). Placentae from PIH (n=7) and NORM(n=9) pregnancies were utilized to isolate cytotrophoblasts via the method of Kliman et. al. (Endocrinol 118:1567;1986). These cells were incubated for 24 hours in culture media with (S) or without (NS) 10% calf serum. Media were assayed for the stable metabolites of thromboxane (TBX_2) and prostacyclin (6-ketoPGF_{1a}) via specific RIA. Separate cultures were incubated with indomethacin 50μM (IND) or arachidonic acid 100μM (AA) to confirm trophoblast prostanoid production. Both TBX_2 and 6-ketoPGF_{1a} concentrations were reduced with IND added and increased with AA added. The prostanoid concentrations are in pg/mg protein. Results: The mean ±SEM concentration of TBX_2 and 6-ketoPGF_{1a} of trophoblasts from PIH patients was less than NORM women.

	TBX_2		PGF_{1a}		ratio	
	S	NS	S	NS*	S	NS
PIH	847±301	875±264	17±5	20±2	55±12	41±12
NORM	1204±164	1299±167	28±5	37±5	42±4	39±7

*p<0.005

Based on these data, cultured cytotrophoblasts in media with or without 10% calf serum from PIH patients have slightly elevated prostanoid ratios (TBX_2 to 6-ketoPGF_{1a}) compared to NORM patients.

65 A COMPARISON OF THE EFFECT OF PHENYTOIN & MGSO4 ON FETAL HEART RATE TRACINGS USING COMPUTER ANALYSIS. E Guzman, M Conley*, R Stewart*, K Kappy, J Ivan*, P Kharabe*, Dept. Obs/Gyn, Newark Beth Israel Medical Center, Newark, New Jersey.

One of the proposed advantages in using phenytoin over MGSO4 for the prevention of eclampsia is its lack of effect on the fetal heart rate tracing. However, this has not been substantiated in the literature. Because of the documented inter- and intra-observer variation in visual interpretation of fetal heart rate tracings (FHRT), we used computer analysis (SYSTEM 8000) to compare the effects of these two medications on the FHRT's of 30 preeclamptic women. Treatment groups were non-randomized and equal in number. FHRT's of 1 hour duration obtained before and after treatment were analyzed in regard to the following parameters: heart rate accelerations of 10 and 15 beats per minute (BPM), episodes of high and low variation, and long and short term variability. No other medications were given during the study period. Using biophysical profile testing along with FHRT, all fetuses were considered well during the control period. Statistical analysis of FHRT parameters before and after treatment were analyzed with paired T-Test and statistical significance was reached at $p<.05$. There was no difference in gestational age, birthweight, APGAR scores, and computer scoring of FHRT's between the control periods of both treatment groups. Phenytoin had no effect on any of the computer measured FHRT parameters. On the other hand, MGSO4 significantly decreased the frequency of accelerations of 10 and 15 BPM, as well as short and long term variability. Though not significant, MGSO4 doubled the amount of time the fetus spent in periods of low variation. Therefore, the use of phenytoin for eclampsia prophylaxis seems preferable to MGSO4 in cases where fetal well-being is in question or FHRT interpretation is difficult, as in prematurity.

66 AN EASILY ADMINISTERED PHENYTOIN REGIMEN FOR THE MANAGEMENT OF PREECLAMPSIA. Michael Lucas, Ralph DePalma, Mark Peters, Kenneth Leveno, David Persons,* F. Gary Cunningham. Dept. Ob/Gyn, Univ. of Texas Southwestern Med. Center, Dallas, Tx.

Current phenytoin regimens for seizure prophylaxis during labor typically involve relatively complicated dosing schemes with varying rates of infusion or multiple intravenous dosages. Moreover, dosage adjustments are recommended based upon body weight. We sought to develop a single regimen that could be used regardless of body weight and that minimized side effects by prolonging the initial infusion. 1 gram of phenytoin was infused over one hour and plasma levels measured in 28 term pregnancies complicated by preeclampsia. In 14 of these pregnancies, phenytoin, 500 mg, was administered orally 10 hours post infusion. The results are summarized below:

Plasma Phenytoin µg/ml Mean (SD) — Elapsed Time (hours) Post Initiation of Phenytoin Infusion

	1	2	8	16	24	32
IV Regimen (N=14)	18(5)	15(5)	11(4)	8(3)	6(2)	5(2)
IV & PO Regimen (N=14)	22(6)	17(6)	13(5)	12(4)	12(4)	11(4)

Side effects were minor and included transient burning at the IV site, mild euphoria, dizziness and nystagmus. A mild and asymptomatic decrease in BP was observed in most women. Comment: Assuming that the therapeutic plasma levels for phenytoin in non-pregnant women (10-20 µg/ml) apply during pregnancy, our regimen of a 1 gram phenytoin infusion followed 10 hours later by 500 mg orally resulted in therapeutic levels for up to 32 hours in all but one woman. This patient weighed 265 lbs. suggesting that only very large women (in excess of 250 lbs.) require dosage adjustment. Moreover, a constant infusion spanning one hour avoids the complexities of adjusting infusion rates or giving multiple intravenous dosages.

67 EFFECT OF LOW DOSE ASPIRIN THERAPY ON POLYUNSATURATED FATTY ACIDS IN PREGNANCY. M.C. McCoy,* H.H.Kay, Y.Wang,* A.P.Killam, Dept. Ob/Gyn, Duke Univ., Durham, NC

Preeclampsia is reported to be due to an imbalance between thromboxane and prostacyclin. Many investigators have found low dose aspirin (ASA) therapy effective in reducing the incidence and/or severity of preeclampsia because it inhibits cyclooxygenase and decreases thromboxane production. We recently reported that plasma levels of polyunsaturated fatty acids in the n-3 and n-6 classes, which are precursors for prostanoids such as thromboxane and prostacyclin, are decreased in preeclamptic patients. The purpose of this study was to determine if low dose ASA therapy affects the plasma levels of these fatty acid precursors, specifically whether these levels would increase with inhibition of cyclooxygenase. We assayed linoleic, linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids using HPLC as previously reported (AJOG 164:812,1991). Eleven pregnant patients at risk for preeclampsia or growth retardation were placed on ASA therapy (81 mg/d) at varying gestational ages. Blood was drawn before starting, after 3-4 days and after 3-4 weeks of ASA therapy. Results were analyzed using the two-tailed paired t-test and sign rank test. We found no significant change in the levels of fatty acids either at 3-4 days or 3-4 weeks compared to baseline. Conclusion: low dose ASA does not appear to change the levels of fatty acid precursors of the prostanoids.

68 CIRCULATING ENDOTHELIN-1 IS NOT INCREASED IN PREECLAMPSIA. D. Shah, M. Frazer,* K. Badr.* Dept. of OB/GYN, The University of Texas Health Science Center, San Antonio, TX and Dept. of Internal Medicine, Vanderbilt University School of Medicine, Nashville, TN.

Endothelin-1 is a novel, potent vasoconstrictor peptide released by vascular endothelial cells. Preeclampsia is a unique hypertensive disorder of pregnancy. Endothelial cell injury has been implicated in the pathophysiology of this disorder. Endothelial cell injury has been shown *in vitro* to cause release of endothelin-1. Therefore, we conducted a prospective study of patients with hypertensive disorders of pregnancy and a control population to examine if circulating levels of endothelin-1 are elevated in these disorders. We studied 21 patients with the following diagnoses: severe preeclampsia ($n=9$); chronic hypertension ($n=6$), of whom two had severe preeclampsia; and control patients ($n=6$). The mean maternal age and gestational ages were similar in these three groups. More importantly, severe preeclampsia patients were selected by very strict criteria and most had evidence of thrombocytopenia. Blood samples were collected in cold EDTA tubes containing aprotinin. Endothelin was extracted immediately after collection of blood and measured by a standard radioimmunoassay. There was no difference between the endothelin-1 levels (mean$\pm$SEM) in the patients with severe preeclampsia (2.1 ± 0.6), chronic hypertensives without superimposed preeclampsia (1.9 ± 0.8), and the control population (1.7 ± 0.6). Our results are consistent with the current concept that endothelin-1 does not function as a circulating hormone. However, because of its prolonged duration of action and function as a locally acting hormone, demonstration of elevated circulating levels of ET-1 is not necessary to support its participation in the vasospasm of preeclampsia.

69 THE EFFECT OF ROUTE OF DELIVERY ON THE IMMEDIATE AND LONG-TERM OUTCOME OF THE VERY LOW BIRTH WEIGHT INFANT IN THE SETTING OF PREECLAMPSIA.

AC Regenstein, RK Laros, A Wakeley[X], JA Kitterman[X], WH Tooley[X], University of California, San Francisco, CA

The role of vaginal delivery of the very low birth weight (VLBW) infant in the setting of preeclampsia is controversial. At our institution, vaginal delivery has been attempted when maternal and fetal factors did not preclude it. An analysis of all singleton liveborn infants weighing 1500g or less and delivered to women with preeclampsia or eclampsia from 1975 to 1990 was undertaken. Of the 116 infants who qualified for the study 54.3% underwent a cesarean section (C/S) without labor. The indications for these C/S were non-reassuring fetal assessment (57.1%), preeclampsia (20.6%) and obstetric contraindications to labor (22.2%). Of the patients allowed to labor 47.2% had a C/S for fetal intolerance of labor and 32.1% delivered vaginally. 73.3% of the patients who delivered vaginally had an unfavorable Bishop score (≤ 5) at the outset of their induction. To investigate the effect of labor on neonatal outcome, the infants who underwent a C/S without labor and with a reassuring fetal assessment were compared to the infants who experienced labor. There were no significant differences between the two groups in gestational age, birthweight, year of delivery, incidence of intrauterine growth retardation (approximately 6.5%), neonatal mortality, incidence of intraventricular hemorrhage, patent ductus arteriosus, or necrotizing enterocolitis. Neurological assessment at follow-up (mean length 4.5 years) and Bayley's score at one year of age were also not statistically different. The incidence of respiratory distress syndrome (RDS) was less in the labor group (50.9% vs. 74.1%, Pearson chi square $p = 0.047$). The incidence of RDS among the infants delivered vaginally was 35.3%. The finding of a lower incidence of RDS in the group that experienced labor and in the vaginally delivered group remained even when controlling for the use of antenatal corticosteriods using logistic regression ($p \leq 0.05$) Based on these limited data a trial of labor is appropriate in carefully selected preeclamptic women who have VLBW infants.

70 PREDICTION OF PREECLAMPSIA BY DOPPLER UTERINE FLOW WAVEFORMS. A. Caruso [X], S. Ferrazzani [X], S. De Carolis [X], G. Rizzo [X], D. Arduini [X] AC. Testa [X], A. Pometti. [X]. Dept. Ob/Gyn, Catholic University, Rome, Italy.

We studied 90 high-risk pregnant inpatients by Doppler ultrasound performed in two periods of gestation: group A (52 pregnancies, 18-24 wks), group B (55 pregnancies, 25-28 wks). 17 patients were studied in both groups. Recordings were obtained using a pulsed color Doppler system at the level of uterine arteries and the higher uterine resistance index (RI) was considered for the analysis. The prevalence of non proteinuric gestational hypertension (NPGH), proteinuric gestational hypertension (PGH), birth weight <2500 g, birth percentile <10th and week of delivery <36 was: 15%, 12%, 35%, 17%, 29% respectively in group A and 24%, 11%, 51%, 18%, 47% respectively in group B. ROC curves indicated that values of RI > 2SD over the mean as cut-off provided the best predictivity. The NPGH plus PGH and birth percentile <10th end-points revealed a low predictivity of the test in both groups. On the other side, the PGH endpoint suggested a good predictive performance: (group A: sensitivity=100%, specificity=78%, positive predictive value [PPV]= 38%, negative predictive value [NPV]=100% and group B: sensitivity=100%, specificity=76%, PPV=33%, NPV= 100%). We emphasize the early onset of PGH (week of delivery 29±3 [mean ±SD]). The birth weight <2500 g and the week of delivery <36 end-points revealed high PPV: 69% and 69% respectively in group A, 95% and 95% respectively in group B. We conclude that color Doppler ultrasound applied to uterine arteries can be a reliable and useful tool in the prediction of both preeclampsia of early onset and poor fetal outcome of high-risk pregnancies. Partially supported by grant 91.00110.PF41 from Progetto Finalizzato FATMA, CNR.

71 CEREBRAL BLOOD FLOW IN PREGNANCY AND PREECLAMPSIA. W Hansen[X], Dept. Ob/Gyn, UNC School of Medicine, Chapel Hill, NC.

Preeclampsia is a hypertensive disorder of pregnancy, associated with a myriad of symptoms and signs, involving numerous organ systems including the central nervous system. Preeclampsia can progress rapidly into a convulsive state termed eclampsia. It is hypothesized that the accompanying neurologic symptoms and subsequent seizures are a result of cerebral vasospasm. The purpose of this study is to determine whether cerebral vasospasm is a concomitant of preeclampsia. Transcranial doppler sonography (TCD), makes it possible to selectively measure the hemodynamic effects of arterial vasospasm, namely increased blood flow velocity. First, cross-sectional groups of healthy pregnant women are enrolled at 6-12 weeks (n=15), 24-40 weeks (n=25), and postpartum (n=15). In each subject, blood flow velocity was measured in the middle cerebral and basilar artery. Normal values are established in each group, and the effect of pregnancy on cerebral blood flow is described. Patients admitted to UNC Hospitals with the diagnosis of preeclampsia (n=18) were enrolled into the study. Serial examinations using TCD sonography of the middle cerebral and basilar arteries were completed from admission through postpartum. Healthy pregnant patients are compared with preeclamptic patients. The relationship between the severity of preeclampsia and vasospasm is examined. Preeclamptic subjects had statistically significant increased cerebral artery blood flow velocities. We conclude that cerebral vasospasm is a concomitant of preeclampsia.

72 ELEVATED ENDOTHELIN IN THE SECOND TRIMESTER IS ASSOCIATED WITH THE RISK OF PREGNANCY INDUCED HYPERTENSION. P. Ogburn, Jr, R. Thompson,[X] A. Lerman,[X] J. Burnett, Jr,[X] M. Cullen, L. Sanchez-Ramos, Mayo Clinic, Rochester, MN & University of FL, Jacksonville, FL.

Endothelin (ET) is a vasoconstrictor peptide which has been shown to be elevated in hypertensive conditions including preeclampsia (PE). In order to determine whether ET elevation may precede clinical evidence of PE, second trimester primigravidas were divided as to positive (+) (n=10) or negative (-) (n=7) roll over tests (ROT). Each of these patients then had angiotensin II infusion tests (AII) with ET measured at baseline and at maximal AII. ET analyses were done using radioimmunoassay. The group with + ROT had 4 of 10 with + AII and 3 of 10 with PIH. The group with - ROT had no + AII and no PIH. ET was 8.87 $\pm$ 0.56 pg/mL in the - ROT and 16.96 $\pm$ 2.59 in the + ROT ($p<.02$). One patient with a + ROT but a - AII developed PIH and had a quite elevated ET of 34.3 pg/mL. AII infusion did not increase the ET levels in any group. Our results suggest that elevated ET is present as early as the second trimester in pregnancies at risk for developing PE.

73 ENDOTHELIN IN ARTERIAL AND VENOUS BLOOD IN SEVERE PREECLAMPSIA.
Arjan A. Kraayenbrink MD*, Gustaaf A. Dekker MD PhD*, and Herman P. van Geijn MD PhD*, Dept. of Obstetrics, Free University Hospital, Amsterdam, The Netherlands.

The endothelial cell is known to release the vasodilators prostacyclin and Endothelium Derived Relaxing Factor, and Endothelin (ET), a potent vasoconstrictor. ET acts mainly as a local hormone. In preeclampsia venous plasma levels of ET are significantly elevated, suggesting that ET acts as an indicator for the severity of the disease. The human lungs are known to remove ± 30% of the circulating amount of ET in one pass. It would be interesting to know if arterial levels of ET are different from venous levels and could act as a better indicator for the severity of preeclampsia. We investigated 6 patients with severe preeclampsia (third trimester) from which both arterial and venous blood was obtained. Six normotensive pregnant women acted as controls for arterial ET levels and 17 other women for venous ET levels. In severe preeclampsia arterial ET levels were higher (mean 11.62, sd 5.52,) compared with venous plasma levels (mean 7.62, sd 1.85). The same result is seen in normotensive pregnancy (arterial: mean 4.82, sd 0.66, n=6; venous: mean 2.28, sd 0.75, n=17). These results show that although ET is substantially removed in the pulmonary circulation, peripheral arterial levels are still higher than venous levels. This suggests continuous arterial production and receptor binding of ET in the peripheral arterial circulation. These findings emphasize the potential role of ET in the pathophysiology of preeclampsia. Urinary ET levels, currently being collected, will provide further insight into the role of ET in preeclampsia.

74 NIFEDIPINE TREATMENT IN PRE-ECLAMPSIA REVERTS THE INCREASED ERYTHROCYTE AGGREGATION TO NORMAL
Tranquilli AL*, Garzetti GG*, De Tommaso G*, Boemi M*, Fumelli P*, Romanini C*. Dept. Obstet/Gynecol, University of Ancona and INRCA, Ancona, Italy

Erythrocyte aggregation was studied by means of an automatic microviscosimeter in 20 normotensive pregnant women, 7 chronic hypertensive, 10 chronic hypertensive with superimposed pre-eclampsia, and 20 women with proteinuric pregnancy-induced hypertension (pre-eclampsia). Erythrocyte aggregation was increased in all the hypertensive pregnant patients, compared to the normotensive pregnant controls, regardless of both the duration (chronic or pregnancy-induced) of hypertension and the status of plasma macromolecules, that did not differ in the groups. Although the antihypertensive treatment with labetalol significantly reduced erythrocyte aggregation, the treatment with nifedipine reverted it to normal. The increased erythrocyte aggregation may be either due to conformational changes of the membrane occurring during hypertension, or to a redistribution of the ionic charges on the two surfaces of the membrane. The specific effect of nifedipine treatment, restoring the ionic charges, may likely be due to this latter event.

75 PLACENTAL LIPID PEROXIDES (LPO) AND THROMBOXANE (TX) ARE BOTH INCREASED AND PROSTACYCLIN (PGI) DECREASED IN WOMEN WITH PREECLAMPSIA.
Y. Wang,* SW Walsh,* HH Kay. Depts OB/GYN, Medical College of Virginia, Richmond, VA and Duke Univ., Durham, NC

There is an imbalance of 1 TX/1 PGI in placentas of women with preeclampsia, but this may not be the only imbalance. Recently reported is an abnormal increase in serum LPO in preeclamptic women (AJOG, Dec., 1991). LPO are toxic compounds that damage cells and inhibit PGI synthesis. The following study examined if LPO were also increased in placentas of preeclamptic women. Placental tissues from 9 normal and 8 preeclamptic women were immediately frozen in liquid N_2 after delivery. Tissue samples (1 gm) were homogenized and analyzed for LPO by MDA and H_2O_2 equivalents, and for TX and PGI by RIA of their stable metabolites, TXB_2 and 6-keto $PGF_{1\alpha}$. LPO were significantly higher in preeclamptic than normal placentas by both analytical methods (MDA: 49 ± 5 vs 31 ± 1 nmol/gm, H_2O_2 equiv: 5.3 ± 0.3 vs 3.2 ± 0.3 µmol/gm, mean ± SE, $P < 0.01$, respectively). TX was significantly higher and PGI significantly lower in preeclamptic than normal placentas (TX: 213 ± 23 vs 158 ± 14 ng/gm, PGI: 24 ± 3 vs 53 ± 7 ng/gm, $P < 0.05$). The ratios of TX/PGI and LPO/PGI were 3-fold higher in preeclamptic than normal placentas. Conclusion: Placental levels of both LPO and TX are increased and PGI decreased in preeclampsia. Speculation: Abnormally increased LPO may be the cause of decreased PGI. HD 20973.

76 MILD PREECLAMPSIA: RARELY A RECURRENT CONDITION.
RK Bhatia, and SF Bottoms. Wayne State Univ., Hutzel Hospital, Detroit, MI.

Chesley reported that 36% of eclamptics develop hypertensive disorders in subsequent pregnancies and speculated that preeclampsia would have a similar recurrence rate. Sibai reported that among women with severe second trimester preeclampsia, 65% develop hypertensive disorders in subsequent pregnancies. Data regarding the recurrence of mild preeclampsia is lacking. This investigation tests the hypothesis that mild preeclampsia is similar in recurrence to more severe disease. We studied 6090 nulliparas who were normotensive prior to pregnancy, received prenatal care, and delivered at our hospital from 1984-1990. A total of 843 (13.8%) had a subsequent delivery at our hospital, with a mean interval between deliveries of 24.2 months. 487 (8%) developed preeclampsia during the first pregnancy, of which 75 (15.4%) had a second delivery. Of the latter, 61 had mild and 14 had severe preeclampsia. Of the 14 severe preeclamptics, 2 (14.3%) had a recurrent hypertensive disorder (severe preeclampsia). Of the 61 mild preeclamptics, 3 (5%) had recurrence (1 each mild, severe, and chronic hypertension). Of 5503 non-preeclamptics, 768 (14.0%) returned for delivery, of which 7 (0.9%) developed a hypertensive disorder in the second pregnancy (6 mild, 1 chronic hypertension). The recurrence risk for mild preeclampsia in this study was significantly lower than rates reported for eclampsia or severe midtrimester preeclampsia (χ^2 goodness of fit, $p < 0.001$ in each case). There was no evidence of followup bias, and diagnostic frequencies are consistent with other large series. The relatively lower recurrence risk associated with mild preeclampsia suggests a lower frequency of preexisting renal/vascular disease and/or less chance of permanent damage secondary to hypertension.

77 RANDOMIZED STUDY OF GENERAL ANESTHESIA VERSUS EPIDURAL OR SPINAL-EPIDURAL ANALGESIA FOR CESAREAN SECTION IN PREGNANCIES COMPLICATED BY SEVERE PREECLAMPSIA. D.H. Wallace,* K.J. Leveno, F.G. Cunningham, V. Shearer,* S. Black,* J. Holloway,* Dept. Anes/Ob-Gyn, Univ. Texas Southwestern Med. Ctr., Dallas, Texas

The choice of anesthesia for cesarean section in pregnancies complicated by severe preeclampsia is controversial. Many clinicians fear the fetal effects resulting from sympathetic blockade secondary to regional analgesia in women with preeclampsia. In this on-going study that was begun in 1989, 66 pregnancies complicated by severe preeclampsia have been randomized to receive either general anesthesia, epidural or spinal-epidural analgesia for cesarean section. Cesarean sections for fetal distress were excluded. Shown below are selected outcomes:

OUTCOME	General	Epidural	Spinal-Epidural	P
Pregnancies	23	20	23	—
Birthweight, g Mean (SE)	1990 (209)	2016 (132)	2621 (185)	.02
Maternal hypotension, ephedrine used (%)	0	6 (30)	5 (22)	.01
Umbilical artery blood pH: ≤ 7.20 (%) ≤ 7.00	1 (4: 0	3 (12) 0	2 (9) 0	NS —
5-Minute Apgar, 3 or less (%)	0	0	0	—

Conclusions: 1) Maternal hypotension is a frequent complication of regional analgesia in pregnancies comp.icated by severe preeclampsia. 2) However, the fetal effects were minimal. 3) Alternatively, general anesthesia was not associated with apparent disadvantages.

78 MATERNAL CHARACTERISTICS FOR EARLY VS. LATE DIAGNOSIS OF GESTATIONAL DIABETES. G.S. Berkowitz[x], R.H. Lapinski[x], S.H. Roman[x], M. Alvarez, C.J. Lockwood. Mount Sinai School of Medicine, New York, NY

A 50 gram glucola test is routinely administered to the patients on our clinic service at the time of their first prenatal visit. If the inital plasma glucose value is < 135 mg/dl, the patien: is rescreened after 24 weeks of gestation. Out of 2776 patients who were screened before 24 weeks between 1986-1990, an abnormal glucose tolerance test was documented in 102 patients before 24 weeks (Early Dx Group) and in 252 patients at 24 weeks or later (Late Dx Group). This study was undertaken to evaluate characteristics associated with early vs. late diagnosis of gestational diabetes (GDM). Univariate comparisons identified the following differences between the Early and Late Dx groups.

Characteristic	Early Dx	Late Dx	P-value
Maternal Age > 30	63.7%	45.2%	<0.01
Preeclampsia	14.7%	7.5%	0.06
Chronic hypertension	11.8%	4.4%	0.02
Body Mass Index ≥ 32.3	28.6%	16.9%	0.02
Weekly Weight Gain < 0.27 kg	45.8%	30.5%	0.01
Prior history of GDM	25.5%	15.1%	0.03
Insulin Use	55.9%	29.8%	<0.01

Logistic regression analysis confirmed that the Early Dx group was significantly older, heavier, more likely to have hypertensive disorders, low maternal weight gain, and a prior history of GDM compared to the Late Dx group. Insulin use during the index pregnancy was also significantly higher in the Early Dx than in the Late Dx group. SUMMARY: These data indicate that a sizeable proportion of GDM patients can be diagnosed early in pregnancy and that differences in maternal characteristics and insulin requirements between the early and late GDM diagnosed groups suggest that the former group may have had preexisting impaired glucose tolerance or diabetes.

79 THE PERINATAL SIGNIFICANCE OF ONE ABNORMAL GLUCOSE TOLERANCE TEST VALUE. R Rait[x], G Berkowitz, M Alvarez, R Lapinski, RL Berkowitz and CJ Lockwood. Mt Sinai School of Medicine, New York, NY.

Gestational diabetes mellitus (GDM) is currently diagnosed by the presence of 2 abnormal values of a 3 hour Glucose Tolerance Test (GTT). However, there have been conflicting reports of the clinical significance of only one abnormal GTT value. We conducted a retrospective study of 5397 clinic patients followed between 1986-89 who were divided into 3 groups: controls with a normal glucose screen (Nls) (n=4868); patients with one abnormal GTT value (Abn-1) (n=176); and those with two abnormal values (GDM) (n=353). The criteria of Carpenter & Coustan were employed (Am J Obstet Gynecol 1982; 144:768-73). Differences in demographic and obstetric outcomes are presented as percents or mean (±SD) and assessed by either chi-square or t-test:

Variable	Nls	Abn-1'	*p+	GDM	*p++
Age > 35 yrs	6.7%	17.6%	0.0001	22.3%	0.02
Nulliparity	45.0%	32.4%	0.0001	28.8%	NS
Hypertension	3.7%	7.4%	0.01	6.5%	NS
Birth Wt ≥90%	10.2%	17.2%	0.003	15.1%	NS
Birth Wt %ile	.52 (.28)	.62 (.26)	0.0001	.56 (.28)	0.04
C-section	16.4%	23.9%	0.008	29.5%	NS

* = comparison of Nls and Abn-1 (p+) or Abn-1 and GDM (p++)

There were no differences in neonatal morbidity including hypoglycemia between the groups. To determine whether the presence of one abnormal value was an independent contributor to the occurrence of macrosomia, a multiple logistic regression was performed. When maternal weight gain, parity, age, smoking and prepregnancy weight were employed as independent variables, no significant association was found between one abnormal value and birth weight ≥ 90th percentile for gestational age.

SUMMARY: Employing a larger number of patients and stricter GTT criteria than previous studies, we note that when potential confounders are controlled for, the presence of one abnormal GTT value is not signficantly associated with macrosomia.

80 RELATIONSHIP OF MATERNAL AND NEONATAL PLATELET COUNT AT DELIVERY. R.F. Burrows, J.G. Kelton[x], McMaster University, Hamilton, Ontario, Canada.

Maternal thrombocytopenia creates anxiety for the obstetrician because of its association with severe neonatal thrombocytopenia. But what is the relationship of these two events? To determine this we prospectively collected maternal platelet counts at delivery and neonatal cord counts over a 63-month interval, recording 11,813 maternal and 12,150 neonatal outcomes. Only 11 of 12,150 infants (0.09%) had severe thrombocytopenia as defined by a cord platelet count $\leq 50x10^9/L$. In contrast, maternal thrombocytopenia ($\leq 150x10^9/L$) was a relatively common event occurring in 789 mothers (6.7%). The maternal platelet count did not predict neonatal thrombocytopenia, with only 4 of the 11 thrombocytopenic infants being born to thrombocytopenic mothers (positive predictive value 0.5%). There were 12 alloimmunized pregnancies resulting in one stillbirth and 6 infants with cord platelet counts $\leq 50x10^9/L$ (6,8,9,11,13,30). Three intrauterine bleeding events were associated with alloimmunization. Thirty mothers who had immune thrombocytopenic purpura (19 with thrombocytopenia) had 3 infants with cord platelet counts $\leq 50x10^9/L$ (21,36,49). There were 1040 hypertensive patients (162 with thrombocytopenia) and 2 of their infants had cord platelet counts $\leq 50x10^9/L$. None of the 590 mothers with thrombocytopenia not in these 3 groups had infants with cord platelet counts $\leq 50x10^9/L$. This study indicates: (a) maternal thrombocytopenia at delivery is not a predictor of neonatal thrombocytopenia; (b) neonatal cord platelet counts $\leq 50x10^9/L$ are rare; and (c) counts $< 20x10^9/L$ and intrauterine bleeding are only associated with alloimmunized pregnancies.

81 **THE SIGNIFICANCE OF ANTICARDIOLIPIN ANTIBODIES IN DIABETIC PREGNANCIES.** *S Rotmensch, EA Reece, M Liberati,* J Peipert,* J Garofalo,* M Breitenstein,* JC Hobbins. Depts OB/GYN and Rheumatology, Yale University*

Anticardiolipin antibody (ACA) is characteristically found in patients with autoimmune diseases and the primary "antiphospholipid antibody syndrome". Gestational and pregestational diabetes are also associated with increases in a variety of autoantibodies. We determined IgG and IgM ACA concentrations in 41 consecutive pregnant diabetics (Classes A, n=21; B, n=13,; C, n=7) using solid phase ELISA (>2SD above mean = positive). Elevated ACA concentrations were found in 32% (13/41; IgM = 2/13; IgG =11/13). No differences were found between ACA positive and negative patients in mean birthweight (3455±573 vs 3369 ± 772gm), gestational age at delivery 39.1 vs 38 wks), mean arterial pressure in all three trimesters (92.6 vs 90.3; 93.3 vs 93.0; 93.0 vs 95.9 mmHg), and platelet count (292,000 vs 287,000 per mm^3). No cases of deep vein thrombosis occurred. One patient in each group was delivered preterm due to early onset severe preeclampsia. Statistical power calculations confirmed adequacy of sample sizes for the detection of clinically meaningful differences. Two intrauterine demises occurred in the ACA negative group. **Conclusions:** Elevated ACA concentrations are frequent in diabetic pregnancies, with an incidence approaching that of patients with systemic lupus erythematosus. However, they are not associated with adverse maternal or fetal outcome.

82 INCREASED PLASMA LEVELS OF ENDOTHELIN-1 IN GRAVIDAS ABUSING COCAINE

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Cocaine abuse during pregnancy is often associated with maternal vascular complications including hypertension and abruption. We investigated the possibility that these complications may be mediated by the vasoactive peptide, endothelin-1 (ET-1). We measured the plasma concentration of ET-1 in 20 patients with acute cocaine intoxication, 20 normal gravidas, and 10 nonpregnant individuals. There were no significant differences in age ($p = 0.64$), gravidity ($p = 0.08$) or diastolic blood pressures ($p = 0.13$) between women using cocaine and healthy, pregnant controls. There were, however, significant differences in the systolic blood pressures (126 ± 18 vs 108 ± 9 mm Hg, $p < 0.01$), gestational age (31.6 ± 3.4 vs 37.5 ± 2.2 weeks, $p < 0.01$), and number of women presenting with evidence of abruption (11 vs 0, $p < 0.01$). No patient met clinical or laboratory criteria for preeclampsia. Plasma concentrations of ET-1 were determined in early labor (cervix dilated ≤ 3 cm) in both pregnant groups using a commercially available radioimmunoassay kit (Amersham). The mean (± SD) concentration of ET-1 in the gravidas with a positive screening assay for cocaine was 12.8 ± 6.2 fmol/ml compared with 5.8 ± 2.7 fmol/ml in the pregnant control group ($p < 0.01$) and 3:5 ± 2.2 fmol/ml in nonpregnant women ($p < 0.01$) The plasma concentration of ET-1 continued to rise in the 5 gravidas with cocaine abuse in whom additional samples were obtained, from a mean of 13.7 ± 6.9 fmol/ml in early labor to 16.2 ± 7.7 fmol/ml at 24 hours after delivery, and remained elevated 48 hours after delivery (13.7 ± 6.6 fmol/ml). In contrast, the concentration of ET-1 in 5 normal gravidas did not change significantly after delivery (5.8 ± 2.5 fmol/ml before delivery, 4.3 ± 0.57 fmol/ml and 3.9 ± 1.1 fmol/ml 24 and 48 hours after delivery, respectively). **Conclusion:** Thirteen of 20 (65%) women who presented with cocaine abuse and pregnancy complications had endothelin-1 levels > 2 SD above the mean for normal pregnant controls. The source(s) of this vasoactive peptide and its role in the pathogenesis of the vascular complications of cocaine abuse remain to be identified.

83 **IS FETAL PULMONARY MATURITY RELATED TO SIZE FOR GESTATIONAL AGE IN PREGNANCIES COMPLICATED BY DIABETES OR HYPERTENSION?** J. Piper,[X] O. Langer, Dept. Ob/Gyn, UTHSC, San Antonio, TX.

Fetal size for gestational age is influenced by maternal diabetes and hypertensive disorders. Alterations in fetal pulmonary maturity have also been attributed to these conditions. Thus, maternal hypertension of sufficient severity to cause fetal growth retardation should also cause the greatest acceleration of pulmonary maturity while the macrosomic infants of diabetic mothers should have the most marked delay of pulmonary maturation when compared to a control population. This hypothesis was tested on all patients undergoing an amniocentesis for maturity studies at this institution since 1986. More than 730 women have been entered thus far in this ongoing study. The associations between birth percentiles, gestational age and lung maturity are summarized below.

PERCENT WITH AN IMMATURE AMNIOCENTESIS

	Preterm (<37 weeks)			Term (≥ 37weeks		
	DM	Htn	Controls	DM	Htn	Controls
SGA	100%	38%	35%	39%	33%	17%
AGA	69%	34%	49%	17%	25%	3%
LGA	42%	–	62%	19%	–	25%
	n=82	*n=43*	*n=331*	*n=147*	*n=7*	*n=126*

This study reveals 1) infants of hypertensive mothers showed no significant acceleration of maturity when compared to controls, 2) preterm AGA infants of diabetic mothers had a significantly higher risk of pulmonary immaturity when compared to hypertensive mothers ($p < .003$) and control mothers ($p < .01$), 3) term AGA infants of diabetic mothers were significantly less mature than controls ($p < .004$) and 4) macrosomic infants of diabetic mothers however, were essentially identical to the LGA infants of women with negative glucose screening, perhaps reflecting a level of glucose intolerance not detected by current screening techniques. In summary, this study showed no effect of hypertension on pulmonary maturity and that the effects of diabetes on fetal size are unrelated to its effects on fetal lung maturity.

84 **DOES MATERNAL DIABETES DELAY FETAL PULMONARY MATURITY?** J.Piper,[X] O. Langer, Dept. Ob/Gyn, UTHSC, San Antonio, TX.

Diabetes in pregnancy has been associated with fetal pulmonary immaturity even at term gestation. Thus, confirmation of maturity has been recommended prior to delivery at ≤39 weeks gestation. We hypothesize that adequate glucose control will prevent the delay in fetal lung maturation as compared to the nondiabetic population. To test this hypothesis, all amniocenteses performed for fetal maturity since 1986 were reviewed and combined with the patients' obstetric data for analysis. To date, 231 diabetic patients and 461 nondiabetic, nonhypertensive patients have been enrolled. Adequate control was defined as mean blood glucose ≤ 105 mg. /dL. (self-monitored). Pulmonary maturity was defined as presence of phosphatidyl glycerol (PG) in diabetics and either presence of PG or Lecithin/Sphingomyelin (L/S) ratio ≥ 2/1 in the nondiabetic patients.

PERCENT WITH AN IMMATURE AMNIOCENTESIS

	Diabetics			Non-
Weeks	x≤105	x>105	Total	Diabetics
<34	90%	89%	91%	68%
34-36.9	43%	47%	51%	27%
37-37.9	26%	38%	32%	7%
38 38.9	8%	20%	15%	13%
≥39	13%	17%	15%	6%

The relative maturity for gestational age was not significantly different in the term diabetic patients than the nondiabetic patients tested. Although there was a trend toward delayed maturity in poorly controlled diabetics beyond 37 weeks, statistical significance was not achieved. In addition, of the 41 diabetic patients delivered within a week of an immature amniocentesis, there were no cases of hyaline membrane disease. In summary, confirmation of fetal lung maturity prior to elective delivery is recommended in all pregnancies prior to 38 weeks. The need for a more aggressive approach to the diabetic patient beyond 38 weeks, cannot be supported by the results of this study.

85 **RANDOMIZED TRIAL OF ELECTIVE INDUCTION VS EXPECTANT MANAGEMENT IN DIABETICS.** OA Henry[A], SL Kjos, M Montoro[X], TA Buchanan[X], JH Mestman[X]. Univ. of So. Calif., Los Angeles, CA

The increased risk of term stillbirth in diabetic pregnancies has promoted elective delivery at term. The purpose of our study was to assess whether the accompanying high cesarean (CS) rate could be reduced. Women with insulin-requiring diabetes in good glucose control, without vascular disease or macrosomia were randomized at 38 weeks to either scheduled induction (IND, N=100) or expectant management (EXP, N=100) with twice weekly ante-partum testing. Induction of labor for obstetrical or medical reasons occured in 49% of the expectant group. Cesarean rates were not different: IND (25%) vs EXP (31%). The mean additional days of gestation age gained by expectant management after study entry was 13 ± 6 (vs 6 ± 4 days in IND). 25 infants in EXP had birthweights > 90th percentile (LGA) vs 15 in IND (p=.05). There were no perinatal deaths, anomalies or differences in neonatal morbidity. In women with uncomplicated insulin-requiring diabetes managed expectantly, only 51% achieved spontaneous labor. Expectant management was not associated a reduction in CS rate or increased morbidity but was associated with more LGA infants.

86 **MANAGEMENT OF PATIENTS WITH INCOMPETENT CERVIX AND BULGING FETAL MEMBRANES.** S.J. Schorr[X], W.J. Morales, Orlando Regional Medical Center, Orlando, FL

During the period of January, 1985 - June, 1991, 45 patients were admitted with a singleton pregnancy < 24 weeks and cervical dilatation ≥ 2.0 cm consistent with a diagnosis of incompetent cervix. Twenty-five had bulging of fetal membranes through the cervix and of these 18 underwent emergency cerclage and the remainder followed expectantly with bed rest. Twenty patients had cervical dilatation with membranes visible at the level of the external os and of these 16 underwent emergency cerclage. The data summarized below indicate a very favorable outcome in patients without bulging membranes. In contrast, although 67% neonatal survivors were achieved by emergency cerclage in those patients with bulging membranes as compared to none managed conservatively, the median duration of pregnancy was 12 (2-94) days resulting in a median gestational age of 25.0 (22-36) week with a documented risk of serious mental and neurological handicaps of 30% at corrected one year of life.

	No	GA	Dilatation	Duration	Survivor
BULGING MEMBRANES					
Cerclage					
Yes	18	22.8	3.2	12 (2-94)	12
No	7	22.8	4.0	2 (1-5)	0
VISIBLE MEMBRANES					
Yes	16	22.3	2.2	50 (30-102)	14
No	4	23.8	3.0	52 (31-74)	4

87 **GESTATIONAL DIABETES AND TIMING OF TREATMENT: IS THERE A DIFFERENCE IN NEONATAL OUTCOME?** O. Langer, E. Xenakis,[x] M. Berkus, A. Samueloff,[x] B. Elliott,[x] Department of Ob/GYN, University of Texas Health Science Center at San Antonio, Texas.

Early in gestation genetic factors dominate changes in fetal growth. The major effects of fetal insulin on delayed or accelerated fetal growth occur late in gestation due to an abnormal (glucose) maternal-uterine environment. In this study, we tested the following hypothesis: In gestational diabetics (GDM) with an abnormal glycemic profile, delayed treatment will result in a higher incidence of macrosomia. 513 GDM women utilizing memory reflectance meters to test their blood glucose 7 times daily and treated under a strict protocol participated in the study. For analysis, they were stratified into early (mean = 27.8±7 weeks' gestation) and late-treated groups (mean=34.5±2 weeks' gestation). The incidence of abnormal fetal growth was:

	Late entry (n=116)	*Early entry (n=397)*	*RR*	*95% CI*
LGA	19.8%	10.1%	2.4	1.4-4.3
SGA	12.6%	8.6%	NS	NS

The study further revealed: 1) in good glycemic control (mean blood glucose <105 mg/dl) late entry patients had 16.7% LGA and early entry patients 4.9% (RR 2.43, 95% CI 1.7-8-7); 2) in contrast, in poorly controlled patients (mean blood glucose >105 mg/dl) the incidence of LGA was similar in both late (28.9%) and early (21.1%) entry groups; and 3) the incidence of LGA/SGA was comparable for the diet only and insulin treated women within both groups. Therefore, prevention of macrosomia requires early timing of treatment as well as good glycemic control.

88 **THE RELATIONSHIP BETWEEN HOME GLUCOSE MONITORING, DATA VERIFICATION AND LEVEL OF CONTROL IN GDM OUTCOME: YOU CANNOT HAVE ONE WITHOUT THE OTHER.** O. Langer, A. Samueloff,[x] M. Berkus, E. Xenakis,[x] Department OB/GYN, Univ. Texas Health Science Center at San Antonio, TX.

Although failure to achieve glycemic control is axiomatically associated with adverse outcome, the majority of studies continue to report a 30% incidence of macrosomia. Controversy also persists regarding the appropriate method of glucose evaluation. We sought to investigate the impact of different methods of glucose assessment on glycemic control and fetal outcome. 2 groups of gestational diabetics (GDM), comparable in age, prepregnancy weight, height and parity were studied Evaluation of Group I (n=1545) was weekly fasting and 2 h postprandial during clinic visits and 4 daily visual glucose strip determinations by the patient. Group II (n=521) utilized memory reflectance meters (7 times daily) to assure accurate and reliable glucose data. The treatment goal in all patients was a blood sugar <105mg/dl.

	Group I	*Group II*	*RR*	*95% CI*
LGA (≥90th %)	27.5%	11.7%	2.86	2.1-3.8
≥4000gm	13.9%	6.3	2.4	1.6-3.5
SGA (≤10th %)	12.9%	12.7%	NS	NS
≤2500gm	6.2%	9.6%	1.6	1.2 2.3

The study further revealed: 1) Group I diet (26.9%) and insulin-treated (28.7%) patients had the highest LGA risk when compared to Group II (RR 4.9, 95% CI 2.10 and RR 5.7, 95% CI 3.3 9.8, respectively); 2) the incidence of SGA was comparable in diet and insulin treated subjects for group I (13.0% vs. 13.5%) and Group II (12.9% vs. 12.3%); 3) despite patient reported near normal mean fasting and 2 h blood glucose for Group I, a 23% incidence of LGA persisted, 36.6% LGA was found in the poorly controlled subjects; and 4) in contrast, in Group II, a 3 fold higher rate of LGA was found in poorly controlled (MBG >105mg/dl) when compared to near normal glycemia (MGB <105mg/dl) 6.6% vs. 21.8%, RR 3.9, 95% RI 2.3 6.8 Because level of glycemia is clearly related to neonatal size it is imperative to verifiy the true level of glycemia, especially when self monitoring of blood glucose is utilized.

89 **GESTATIONAL DIABETES AND INFECTION: AN INFANT AND NOT MATERNAL COMPLICATION.** O. Langer, M. Berkus, A. Samueloff, E. Xenakis,[x] N. Field,[x] Dept. OB/GYN, University of Texas Health Science Center at San Antonio, Texas.

Diabetes in non-pregnant women (characterized by severe glucose abnormality) has been associated with a high incidence of infection and resulting morbidity. In contrast, there is a dearth of information regarding gestational diabetes (GDM) represented by milder glucose abnormality and infection. Therefore, we sought to investigate thea relationship between GDM and infection. 1740 consecutive gestational diabetic women and 24500 non-diabetic controls were randomly selected from our data base. The 2 groups were comparable in infection-related factors such as maternal age, parity, duration of labor, time from rupture of membranes to delivery and incidence of internal FHR monitoring. To control for method of delivery, each of the 2 groups was stratified into vaginal (VAG), instrumental (INSTR) and cesarean section (C/S) and measured for infection variables.

	DIABETIC (n = 1740)			*NON-DIABETIC (n = 24500)*		
	VAG	*INSTR*	*C/S*	*VAG*	*INSTR*	*C/S*
Neonatal infect	4.0	12.3	8.4	4.0	7.2	10.0
Maternal infect	2.5	3.7	23.6*	2.1	4.0	32.3
Endometritis	2.4	3.7	19.0*	2.0	4.6	26.8
Wound infect	0.1	0	4.6	0.1	0.2	5.5
Chorioamnionitis	1.5	2.5	3.8	1.1	2.3	4.8
Pylonephritis	8.3	8.6	13.5*	7.9	9.4	9.3

*Significant ($p < .01$) between the groups for a given category; all others N.S.

The study further revealed: 1) when augmentation subjects were compared to spontaneous vaginal deliveries, a 3-fold higher risk for maternal infection was found in both groups; 2) the incidence of fetal distress, 5-min Apgar score <7, neonatal death and pneumonia were significantly higher in diabetic fetuses with infection when compared to non-diabetic fetuses; and 3) within each group, fetal morbidity and mortality was significantly higher in the infection population. Pregnant diabetics are not at higher risk to develop infection in comparison with the general population. In contrast, the maternal disease may be the catalyst for fetal infection and the resultant morbidity.

90 **PLATELET AGGREGABILITY IN PREGNANT DIABETICS IS CORRELATED WITH GLYCEMIC CONTROL BUT NOT DURATION OF DISEASE.** RD Jelsema[x], MC Eustice[x], SF Bottoms, and EF Mammen[x]. Wayne State Univ./Hutzel Hosp., Detroit, MI

Platelet hyperaggregability among non-pregnant diabetics has been demonstrated using whole blood aggregometry. This could be due to vasculopathy, but we have recently reported evidence suggesting glycemic control may be responsible. To clarify the roles of vasculopathy and glycemic control, we studied 14 non-diabetic pregnant controls, 7 insulin dependent gestational diabetics (GDM), and 24 diabetics with disease predating pregnancy (IDDM, mean duration 9.1 years). Platelet aggregability and ATP release in whole blood in response to ADP, collagen, and arachidonic acid were measured using a Chronolog® Lumi-aggregometer. Glycated hemoglobin was measured and glycemic control was categorized as good, intermediate, or poor based on fasting blood sugar. The GDM and IDDM groups had higher platelet aggregability than pregnant controls (MANOVA, $p<0.005$). There was no difference in aggregability between GDM and IDDM, and no significant relationship to the duration of diabetes. Among diabetics, canonical correlation of glycated hemoglobin and glycemic control to ATP release in response to ADP and collagen accounted for 34% of total variance ($p<0.005$). These findings are consistent with a prior study demonstrating increased platelet clumping in whole blood with the addition of glucose that was inhibited by apyrase, an ADP removing enzyme. We conclude that platelet hyperaggregability among pregnant diabetics is related primarily to glycemic control rather than duration of diabetes, which is in turn related to vascular disease.

91 **AFFINITY CHROMATOGRAPHY: EXCELLENT CORRELATION WITH HgbA1c.** RD Jelsema[x], MP Dombrowski, and SF Bottoms. Wayne State Univ., Hutzel Hospital, Detroit, MI.

Counseling diabetics for risk of fetal anomalies is based exclusively on studies using older techniques such as ion exchange column HgbA1c (intra- and inter-assay variation 4% and 6%). Newer affinity chromatography (AC) methodology has the following advantages: 1) greater precision (intra- and inter-assay variation < 3%), 2) measures all glycated Hgb, not just glycated A or A1c, 3) does not measure nonglycated Hgb variants S,C,F, and 4) is faster and less expensive. Correlation of these two methods in pregnant diabetics has not been reported. We prospectively performed both tests on blood samples from 83 pregnant diabetics over a period of 6 months. Using only the first determined level for each patient, and excluding all patients with hemoglobinopathies, 78 samples remained. The mean gestational age (GA) was 21 weeks, with 11 samples from the 1st trimester. Stepwise regression analysis was performed with HgbA1c as dependent, and GA and AC as independent variables. AC was highly correlated with HgbA1c ($r = 0.92$), regardless of GA, which had no significant effect. The regression equation was: HgbA1c = 0.66 x AC + 2.7. Using this formula, Ylinen's critical HgbA1c of 10% corresponds to an AC of 11%. Based on these results and the other advantages of AC outlined above, we recommend its use in diabetic pregnancy.

92 **EFFECT OF SUBSTANCE ABUSE REPORTING LAWS ON COCAINE USE IN SUBSEQUENT PREGNANCIES**
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Since 1987, all women at Hennepin County Medical Center delivering babies exposed to cocaine during pregnancy have been reported to the county Child Protective Services, for assessment, intervention and optional chemical dependency therapy. In 1988 and 1989, 176 women or their babies tested positive for cocaine at delivery. When these women presented for prenatal care in a subsequent pregnancy, they were prospectively identified and followed by social services and obstetrics for evidence of continued substance abuse. As of August 15, 1991, 43/176 women had completed another pregnancy at our institution. Maternal charts were reviewed and demographic data as well as pregnancy outcome were recorded. 3/45 women (7%) had no known substance abuse in pregnancy. 23/45 (51%) again tested positive at delivery for cocaine. 15/45 (33%) tested negative for cocaine at delivery but had documented use in pregnancy and 4/45 women (9%) had no evidence of cocaine use in the subsequent pregnancy but had documentation of heavy alcohol use. Of the 42 non-sober women, 15/42 (36%) delivered babies weighing <2500 grams, 15/42 (36%) delivered prior to 37 completed weeks gestation, 6/42 (14%) had no prenatal care and 31/42 (74%) had <7 prenatal visits. 10/23 (43%) positive at delivery had participated in some form of voluntary chemical dependency treatment since their previous delivery. 4/15 women who had used cocaine in pregnancy but were negative at the time of delivery were active in treatment programs at the time of delivery. We regretfully conclude that notification of child protective services and offering optional chemical dependency treatment does not appreciably alter substance abuse in future pregnancies. States considering implementation of mandatory substance abuse reporting laws such as the 1989 Minnesota law should consider this information. A more intensive program of child protective intervention including use of court-ordered chemical dependency treatment is now being implemented and its impact on recidivism must be assessed.

93 MATERNAL AND PERINATAL OUTCOME OF 18 CASES OF ADULT RESPIRATORY DISTRESS SYNDROME (ARDS) IN PREGNANCY.

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There is limited information available regarding maternal and perinatal outcome in pregnancies complicated by ARDS. The purpose of this clinical investigation is to report our experience with ARDS in patients managed in an Obstetric ICU at a tertiary care center. Patients and Methods: The study population consisted of 18 patients who developed ARDS in pregnancy or immediately postpartum in a 6-year period during which there were 47,200 deliveries. Results: The incidence of ARDS was 1 in 2,662 deliveries. Infection was the most frequent cause of ARDS (11/18, 61%) including pneumonia (4 varicella, influenza A, and pneumococcus), pyelonephritis (n=4), and chorioamnionitis (n=1). Other causes of ARDS were preeclampsia-eclampsia (n=3), massive hemorrhage (n=2), TTP (n=1), and smoke inhalation (n=1). Fifteen of 18 patients required mechanical ventilation (mean 13.5, range 3-54 days). Pneumothorax occurred in 7 patients; multiple organ failure developed in 10 patients. Maternal mortality was 39%. There was 1 ectopic pregnancy and 2 spontaneous second trimester abortions. Of 15 pregnancies that reached viability (≥24 weeks' gestation), 4 fetuses died (fetal distress secondary to maternal shock, trisomy 18, abruptio placenta, and uterine rupture). Of 11 surviving infants, 9 did well and 2 had major morbidity. Outcome of the 15 patients requiring mechanical ventilation is shown in the table below. Conclusions: ARDS is an uncommon complication of pregnancy with diverse etiologies and substantial morbidity and mortality. Multiple organ failure is associated with poor maternal and perinatal outcome.

	Respiratory Failure Only (n=5)	Multiple Organ Failure (n=10)
Maternal death #(%)	1 (20)	6 (60)
Days ventilation (mean)	6 (range 3-9)	17 (range 3-54)
Perinatal deaths #(%)	1 (20)	2/7 (29)*
Neonatal morbidity	0	2/5 (40)

*1 ectopic, 2 spontaneous abortions

94 PREECLAMPSIA IN HYPOTHYROID PREGNANCIES.

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The purpose of this study was to investigate the relationship between hypothyroidism and preeclampsia. A cohort of 97 hypothyroid pregnant patients were evaluated in our medical specialty clinic between 1978-1990. Patients with any other medical complications were excluded. Initial and subsequent thyroid function studies were obtained. Overall, the incidence of preeclampsia was 12.3% (12/97). Comparison was made between the patients with (P) and without preeclampsia (no P).

	P(N=12)	no P(N=85)	Pvalue
age	26.9	29.7	N.S.
parity	1.6	1.6	N.S.
initial FT4I	5.7	7.5	N.S.
initial TSH (mIU/ml)	72.4	24.5	0.0038
FT4I prior to delivery	6.2	10.0	0.0002
TSH prior to delivery (mIU/ml)	41.5	5.5	0.0000
No. positive antimicrosomal antibody	2	37	N.S.
No. positive antithyroglobulin antibody	2	26	N.S.
No. positive TSH receptor antibody	1	8	N.S.
No. never euthyroid during pregnancy	8	20	0.006

Preeclampsia was more likely to occur in patients who were more hypothyroid on presentation and remained hypothyroid at delivery. The presence of antimicrosomal, antithyroglobulin, and TSH receptor antibody was not a significant factor. The correction of hypothyroidism is essential in the management of these patients.

95 CHALLENGING THE NORMAL GLUCOSE CHALLENGE TEST: Zion Hagay[X], Gil Bolotin[X], Roni Levy[X], Vaclav Insler[X], E. Albert Reece*, Departments of Ob/Gyn, Kaplan University Hospital, Israel and Temple University School of Medicine, Philadelphia, PA*

The most widely used screening test for gestational diabetes is the 50 gr oral glucose load. A threshold value of 140 mg/dl or higher is recommended for use as a positive test, requiring a 3-hour oral glucose tolerance test. However, it remains unclear whether minimal glucose elevation even in this normal range is associated with perinatal complications. The purpose of the present prospective study was to determine whether a single glucose challenge test (GCT) result, currently considered to be in the normal range is associated with pregnancy complications. MATERIALS AND METHODS: A total number of 225 consecutive pregnant patients who underwent 50 gr oral GCT and who had negative test results (plasma glucose level of 139 mg/dl or less at 1 hour) were evaluated. All 225 patients were identified based on the following criteria: 1. Plasma glucose determinations performed at 24-28 weeks gestation. 2. Patients had one or no previous deliveries. 3. No previous history of cesarian section or gestational diabetes or any medical disorders. The 225 patients were divided into two groups according to their blood glucose level, Group A (n=221), women with plasma glucose levels less than 120 mg/dl, Group B (n=54), those with plasma glucose level of 120-139 mg/dl. RESULTS: Mean birth weight was not significantly different between the two groups (3,252±410 gr vs. 3,304±382 gr). Patients with elevated GCT, although in the normal range, had a significantly higher rate of cesarean section, preeclampsia or both (12.2% and 31.2% in Group A and B respectively) ($p<0.05$). Furthermore, minor congenital anomalies were also significantly ($p<0.05$) higher in Group B (9.3%) than in Group A (4%). CONCLUSION: The results of this study demonstrate that even mild degrees of hyperglycemia at the time of GCT can be associated with higher rates of adverse pregnancy outcome. Hence, the present data challenge the utility of the current threshold value for the GCT.

96 ACCELERATED FETAL GROWTH FOLLOWING EARLY GROWTH DELAY IN INSULIN-DEPENDENT DIABETIC PREGNANCIES.

T.A. Siddigi, B. Rosenn, J. Khoury,* M. Miodovnik, Dept. Ob/Gyn, Univ. Cincinnati Med. Ctr., Cincinnati, OH

Although macrosomia is a common complication in infants of diabetic mothers (IDMs), early fetal growth delay has been observed in insulin-dependent diabetic (IDD) pregnancies. This prospective longitudinal study was designed to establish fetal growth characteristics of IDMs compared to normal controls. Twenty-five IDDs and 32 normal, non-diabetic controls were recruited prior to 12 weeks' gestation. Gestational age (GA) was established by menstrual history and first trimester sonogram and confirmed at birth by physical examination. Patients in both groups underwent a complete ultrasound examination of the fetus during the first trimester and every 4 weeks from 20 weeks' gestation until delivery. Growth curves of the biparietal diameter (BPD) and abdominal circumference (AC) were established for fetuses in both groups. A biphasic growth pattern of the BPD was found in the IDM group, described by the cubic equation: $BPD = 9.64 - 1.01\ GA + 0.05\ (GA)^2 - 0.00068244\ (GA)^3$. BPD growth in the control group was described by a different cubic equation: $BPD = 5.769 - 0.5957\ GA + 0.03826\ (GA)^2 - 0.000532\ (GA)^3$. Compared to the growth pattern of normal controls, IDMs demonstrated early BPD growth delay followed by accelerated growth starting early in the third trimester. The AC growth pattern was linear in both groups, with a steeper slope in the IDMs. Thus, despite initial growth delay of AC in the IDM group, catchup with the control group was evident by the middle of the third trimester. Our findings support the suggestion that fetal growth in IDD pregnancies is biphasic, characterized by a phase of early delay followed by accelerated growth; however, the mechanisms underlying this phenomenon are yet to be determined. We speculate that the initial toxic effect of hyperglycemia causing stunted growth of the embryo is later followed by the effects of hyperinsulinism, once the fetal pancreas maturates and becomes metabolically active.

97 MATERNAL-PERINATAL OUTCOME IN WOMEN WITH CARDIAC DISEASE.

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The purpose of this study is to report maternal and perinatal outcome in pregnancies complicated by maternal cardiac disease. The study population included 54 women with documented cardiac disease prior to the onset of pregnancy. Eight women had various cardiac arrhythmias, 5 had mitral valve prolapse. These 13 women had 14 pregnancies at term without maternal complications. Three women had aortic insufficiency; 1 developed pulmonary edema during labor. Two women had tetralogy of Fallot; both pregnancies resulted in live full term babies; however, 1 patient developed postpartum left middle cerebral artery embolus. Two patients with myocardial infarction delivered 3 liveborn infants without any maternal complications. Pregnancy outcome in the remaining 30 women is summarized in the table. Conclusions: proper management of women with cardiac disease results in good maternal/perinatal outcome. However, women with tricuspid atresia and/or pulmonary hypertension and cardiomyopathy remain at increased risk for maternal morbidity and mortality.

	ASD/ VSD n=15	Aortic/ pulmonic stenosis n=8	Tricuspid atresia Pulm.HTN n=4	Cardio- myo- pathy n=7
No. of pregnancies	21	12	4	9
Deliv. ga (wk)	37.5±3.6	38.5±2.7	35.3±2.3	37.8±3.5
Preterm<37 weeks	4(19)	2(16)	2(50)	2(22)
Birthweight (g)	3015±775	3134±540	2218±646	2732±782
<10th percentile # (%)	1 (5)	1 (8)	2 (67)	3 (33)
Perinatal deaths # (%)	0	0	1(25)*	0
Maternal deaths # (%)	0	0	2(50)	1 (9)**

ASD=atrial septal defect, VSD=ventricular septal defect, *=1 had pregnancy termination, **=died 2 months postpartum.

98 PERINATAL OUTCOME AND DIABETES MELLITUS

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125 insulin dependent patients were cared for during their pregnancies according to the following protocol: Diet: 24-30 calories/kg b.w., 20% as protein, 50% as CHO, 30% as fat, 3 meals and 3 snacks/day. Insulin 0.5-1.0 u. Reg insulin/kg b.w. with 40% before breakfast, 30% before lunch, 20% before supper and 10% at bedtime. 2-4 u. NPH h.s. Exercise: aerobic at least 3 times/week. Glucose determinations: fasting and 2-hour post prandials at least 3 times/week. Goal: Maintain FBS and 2-hour p.p. below 150 mgm/dl. Results: Mean b.g. by trimesters-first: 143 mgm/dl 2d:131 3d:131. Mean FBS-144 mgm/dl, post breakfast-146, post lunch-116, post supper-125. B.W. >4 kg-11%. Neonatal hypoglycemia-9%. Incidence of Iga with mean b.g. 120:17%, with b.g. 120-150: 30%, with b.g.>150:30%. Perinatal mortality: 3.1%, due to anomalies: 3/7 (43%).

Conclusion: 150 mgm/dl is an adequate endpoint with acceptable perinatal mortality/morbidity and a low incidence of maternal hypoglycemia. The size of the neonate is significantly lowered only if the maternal b.g. is kept below 120 mgm/dl. Preconceptional control seems mandatory to minimize congenital malformations and perinatal mortality.

99 PERINATAL OUTCOME IN PREGNANCY COMPLICATED BY MASSIVE OBESITY

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The purpose of this study was to evaluate the impact of massive obesity on pregnancy outcome. Between 1/1/86 and 12/31/90, women weighing >300 lb at delivery (n=111, 0.43% incidence) comprised the study group (G1). To control for confounding variables, another group (G2) was comprised of the massively obese patients without the complications of diabetes and/or hypertension. A control group (CG, n=112) matched for maternal age and parity, was consecutively selected following each case delivery. Perinatal outcome was analyzed between groups and is demonstrated below.

Outcome	%G1 n=111	%CG n=112	%G2 n=86	P values G1 v CG	G2 v CG
1° Cesarean	32.4	15.1	19.5	.0004	NS
BWT > 4kg	30.2	11.3	14.6	.0005	NS
BWT < 2.5kg	14.7	3.8	3.7	.008	NS
IUGR	8.1	0.9	4.9	.03	NS
NICU ADMIT	15.5	4.7	4.9	.01	NS
Preeclampsia	4.5	0.0	4.9	.07	NS
Class B DM	19.8	2.7		.00006	
Class A DM	5.4	0.9		NS	
Hypertension	27.0	0.9		.0001	

Conclusion: Massively obese patients without confounding medical complications had a pregnancy outcome similar to controls. These data may be useful in preconceptual and prenatal counseling, and in perinatal management of the massively obese patient.

100 SEVERITY OF ASTHMA AND PREGNANCY OUTCOME: A CASE CONTROL STUDY

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Between 1/1/81 and 12/31/90, 183 patients delivered who had the diagnosis of asthma (incidence 0.59%). 81 patients were identified as requiring medication control (ie steroids, theophylline, betamimetics, etc.) throughout pregnancy. These patients included 50 non-steroid dependent asthmatics (NSA) and 31 steroid dependent asthmatics (SA), and were compared with a group of 130 randomly selected patients (CG), excluding maternal transports. All three groups were similar for BWT <1500 gm, low 5 minute Apgar, IUFD, hypertension, preeclampsia, and anomalies. The remainder of perinatal outcome analysis is demonstrated in the table below:

Outcome	%SA n=31	%NSA n=50	%CG n=130	P values SAvNSA	SAvCG	NSAvCG
BWT <2500 gm (LBW)	45.2	14.0	4.6	<.01	<.01	.06
Deliv. <37wk (PTD)	54.8	14.0	3.9	<.01	<.01	.03
Preterm Labor (PTL)	48.4	10.0	1.6	<.01	<.01	.03
PROM	25.8	10.0	1.6	NS	<.01	.04
C/S Distress	16.1	14.0	1.6	NS	<.01	<.01
Class A_2 Diabetes	9.7	4.0	.78	NS	.03	NS
Class B Diabetes	6.9	2.0	0	NS	.04	NS

These data suggest that medication dependent pregnant asthmatics are at increased risk for perinatal morbidity. Risk factor counseling, preterm labor prevention and assessment, and early screening for diabetes should be considered.

101 OUTCOME OF PREGNANCY IN PATIENTS WITH PREEXISTING RENAL DISEASE. TK Sorensen,[X] TR Easterling, TJ Benedetti. University of Washington Medical Center, Seattle.

Thirty pregnancies in 27 patients with chronic renal disease were reviewed. Chronic renal disease was defined as serum creatinine ≥ 1.2 mg/dl or creatinine clearance < 90 ml/min during pregnancy or urine protein > 3 gm/24 hours. 5 patients had severe disease with serum creatinine > 2.5. Cardiac output was measured by Doppler technique.

Low birth weight (<2500 gr)	63%
premature (<37 wks)	63%
growth retarded (SGA)	44%
Chronic hypertension	92%
Preeclampsia	50%
Fetal distress	40%
Cesarean section	53%
Perinatal loss (after 2nd trimester)	17%
Anomalies	3%

Five of 6 (82%) diabetics had markedly elevated cardiac output in contrast to an underlying hemodynamic pattern of elevated total peripheral resistance in 60% of the remaining patients; pregnancy outcomes in the diabetics were similar to the group as a whole. Three of the 5 patients with severe disease had detioriation of disease requiring dialysis during pregnancy. In conclusion, our patients did not appear to have an increased rate of fetal anomalies, but were at high risk for prematurity, growth retardation, fetal distress, operative delivery, and preeclampsia. Renal function worsened only in patients with severe disease.

102 GESTATIONAL DIABETES SCREENING: RELATIONSHIP OF NON-FASTING PLASMA GLUCOSE AND 50 GM GLUCOSE CHALLENGE TEST VALUES TO TIME FROM THE LAST MEAL. Sermer M[X], Naylor CD[X], Farine D, Cohen H, Ritchie JWK, Gare D[X], Kenshole A[X], McArthur K[X], Biringer A[X], Holzapfel S[X]. University of Toronto Perinatal Complex, Toronto, Ontario, Canada

Background: Use of Non-Fasting Plasma Glucose [NFPG] as a screening test for Gestational Diabetes [GD] has been suggested, but variability in relation to meals is a concern. Glucose Challenge Test [GCT] has the advantage of a standard glucose load but might also be variable among non-fasting subjects. Objective: Assess influence of time since last food ingestion (to nearest hour) on NFPG and non-fasting GCT values. Setting: Three Toronto teaching hospitals. Subjects: 1318 consenting, consecutive patients, age 24 and over had NFPG and GCT at 26 wks gestational age. Time from the last meal was obtained. Of these 1199 (91%) went on to Oral Glucose Tolerance Test. National Diabetes Data Group criteria was used to define GD. Results: Mean age was 31 yrs with median G2-P1 status. Mean NFPG and GCT results (mmol/L) are shown by time since last meal.

Time	GCT	SE	NFPG	SE
1	6.61	0.091	5.11	0.058
2	6.41	0.077	4.73	0.047
3	6.39	0.090	4.54	0.047
4	6.89	0.152	4.32	0.065
5	7.02	0.131	4.28	0.043

ANOVA showed highly significant (p<0.0001) time-effects in both cases. For NFPG, using Tukey's studentized range test, all pairwise differences were significant at p<0.05 except 3v. 4h. and 4v. 5h. For GCT, the non-monotonic influence of time meant that only the 5v. (1,2,3) and 4v. (2,3) comparisons were significant.

Conclusions: Both NFPG and non-fasting GCT are highly influenced by time from the last meal. The monotonic relationship for NFPG contrasts strikingly with the J-shaped curve for GCT. Although sample size is still limited for proving definitive differences, ROC curve analysis supports the hypothesis that cut-point for non-fasting GCT should vary according to the time since the last meal. Analysis of additional 1700 patients will be completed by December 1991. Continuation to 4000 subjects is planned.

103 GESTATIONAL DIABETES SCREENING TEST PERFORMANCE REVISITED: INTERIM INSIGHTS FROM A PROSPECTIVE STUDY. Sermer M[X], Naylor CD[X], Farine D, Cohen H, Ritchie JWK, Gare D[X], Kenshole A[X], McArthur K[X], Biringer A[X], Holzapfel S[X]. University of Toronto Perinatal Complex, Toronto, Ontario, Canada

Background: Not since work by O'Sullivan have screening tests for Gestational Diabetes [GD] been fully appraised by attempting to administer a 100 gm Oral Glucose Tolerance Test [OGTT] to all subjects regardless of screening test results. Objective: Assess and compare GD screening performance of Non-Fasting Plasma Glucose [NFPG] and 50 gm Glucose Challenge Test [GCT], using National Diabetes Data Group criteria. Setting: Three Toronto teaching hospitals. Subjects: 1318 consenting, consecutive patients, age 24 and over had NFPG and GCT at 26 weeks gestational age. Of these 1199 (91%) went on to OGTT; they were similar to non-compliant subjects and the general obstetrical population. Results: Mean age was 31 yrs, with median G2-P1 status. Incidence of GD was 4.3%. Results (in %) are shown by NFPG and GCT cut-points (mmol/L):

GCT	6.8	7.0	7.2	7.4	7.6	7.8	8.0
Sens.	90	88	80	80	77	77	75
Spec.	58	65	69	73	77	79	82
PPV	9	10	10	12	13	15	17
NPV	99	99	99	99	99	99	99

NFPG	4.0	4.2	4.4	4.6	4.8	5.0	5.2
Sens.	94	92	82	78	65	63	51
Spec.	18	31	44	55	63	71	77
PPV	5	6	6	7	7	9	9
NPV	99	99	99	98	98	98	97

Conclusions: Balancing false positive [FP] and false negative [FN] rates, NFPG is clearly inferior to GCT. However, even the GCT has a large and significant 23% FN rate (1-sens.) with the standard 7.8 mmol/L cut-point. PPV of GCT and NFPG is poor, due to low prevalence and a high FP rate. Sample size is too small to rule out equivalence of NFPG and GCT, once NFPG is adjusted for time from the last meal, but current data are not promising. Analysis of additional 1700 patients will be completed by December 1991. Continuation to 4000 subjects is planned.

104 A COMPARISON OF PTU VERSUS TAPAZOLE IN THE TREATMENT OF HYPERTHYROIDISM

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We compared the efficacy of PTU and Tapazole in the treatment of hyperthyroidism in pregnancy. Between 1974 and 1990, 153 hyperthyroid patients were medically managed in our clinic. A total of 129 charts (84%) were retrieved. Of these, 95 patients were treated with PTU and 34 were treated with Tapazole. The time to remission with PTU and Tapazole was not statistically different. Twenty six of the 95 (27%) treated with PTU remained hyperthyroid; while 9 of the 34 patients (26%) treated with Tapazole remained hyperthyroid. Treatment with PTU or Tapazole reflecting a euthyroid state decreased the incidence of LBW infants equally in both groups. PTU was discontinued in two patients secondary to rash. The incidence of major congenital malformations was 3% in both groups. One tapazole exposed infant had an inguinal hernia. The anomalies seen with infants exposed to PTU included: VSD, pulmonic stenosis, and a PDA in a term infant. No scalp defects were seen in Tapazole exposed infants. In summary both PTU and Tapazole are equally efficacious in treating hyperthyroidism and have comparable fetal outcomes.

105 **LEFT VENTRICULAR (LV) SYSTOLIC (S) AND DIASTOLIC (D) FUNCTION IN PREGNANT PATIENTS WITH SICKLE CELL ANEMIA (SCA).** J.C. Veille, R. Hanson, Dept. of Ob/Gyn, Bowman Gray School of Medicine, Winston-Salem, NC

Patients with SCA may have a cardiomyopathy secondary to chronic anemia. The cardiovascular stress of pregnancy may compromise LVS and D function. Echocardiograms were done on 13 SCA (SS and SC) patients and compared to 20 normal pregnant patients. All studies were done in the third trimester (X GA 34 ± 2 weeks), with the patients in the left lateral decubitus. Results are expressed as X and SD; ANOVA test was used to determine statistical analysis.

HRs = 84.6 ± 11	EDDs = 52.1 ± 6	LAs = 38.4 ± 6
HRc = 87.6 ± 12	EDDc = 48.8 ± 4	LAc = 31.4 ± 6
p = NS	p < 0.02	p < 0.005
FSs = 38.6 ± 7	SVs = 88.5 ± 2.4	DDDTs = 11.7 ± 2
FSc = 37.3 ± 6	SVc = 66.6 ± 2.0	DDDTc = 9.0 ± 2
p = NS	p = 0.002	p = 0.001

(Legends: EDD=end diastolic dimension (mm); LA=left atrium (mm); FS=fractional shortening (%); SV=stroke volume (ml); DDDT=diastolic relaxation); s=sickle cell; c=control. Results: 1) No difference in HR and FS were found between the two groups; 2) Left ventricular and atrial dilatation was present in the SCA group; 3) Stroke volume was significantly increased in SCA; 4) Left ventricular relaxation was abnormal in the SCA group. Conclusions: In the presence of a chronic medical condition which decreases oxygen-carrying capacity, pregnant patients with SCA have a greater cardiac dilatation than the pregnant control. Even though systolic function is preserved, diastolic stiffness may compromise cardiac function in strenuous periods, one of those being active labor. (Supported in part by NIH Grant HL38296).

106 **DOES VARIATION EXISTS BETWEEN 100 GM VS. 75 GM OGTT?** L. Brustman,[x] B. Gela,[x] M. Moore,[x] K. Reilly,[x] O. Langer.[*] Depts. OB/GYN, Our Lady of Mercy Medical Center, Bronx, New York, *University of Texas Health Science Center at San Antonio, Texas.

Recently it was proposed (3rd International Workshop on Gestational Diabetes-Chicago) that the 75gm challenge test should replace the traditional 100gm OGTT load. Paucity of data exists regarding the variation between the 2 tests, translation criteria and association to fetal outcome. We set out to investigate, in an ongoing study, the variability in 75gm oral glucose tolerance test in comparison to 100gm OGTT. 23 subjects participated in the study in which each patient served as her own control performing 75 and 100gm OGTT within 1 week. Plasma glucose levels were measured by the Beckman Autoanalyzer which has been shown to have high (99%) test/retest reliability. The results are shown in the table below:

	100g OGTT	*75g OGTT*	*P*	*r (100g vs 75g)*
Gestational age (wks)	30.7 ± 3.0	31.1 ± 3.2	NS	
Fast (mg/dl)	88 ± 16	80 ± 16	.006	0.72
1 hour (mg/dl)	179 ± 47	169 ± 45	.01	0.92
2 hour (mg/dl)	159 ± 45	141 ± 42	.02	0.72
3 hour (mg/dl)	118 ± 42	97 ± 33	.002	0.75

9/23 women were diagnosed as gestational diabetics (GDM) using the modified O'Sullivan criteria (NDDG, 100gm load, ≥ 2 abnormal values). In contrast, only 4 of these 9 would have to be identified by the 75gm load using the same threshold in diagnostic criteria (as the 100gm load). Furthermore, the 5 subjects that were not diagnosed as GDM had 1 abnormal value on their test results. Our data suggest that despite the strong association between the 2 glucose loads, significant variation exists in the test results. Thus in order for 1 test to approximate a mirror image of the other, new thresholds of glucose abnormality need to be developed.

107 FETAL SEX AND HYPEREMESIS GRAVIDARUM. C.D. Hsu*, Frank R. Witter, Dept. Gyn/Ob. The Johns Hopkins University School of Medicine, Baltimore, MD.

Background: The cause of hyperemesis gravidarum remains largely undetermined and debated. It has been suggested that elevated human chorionic gonadotropin (hCG) or estradiol level is associated with hyperemesis gravidarum. A fetal sex related effect on hCG concentration in singleton and twin pregnancies has also been reported. Pregnant women carrying singleton female fetuses or twin female-female, female-male fetuses have higher hCG levels than male fetuses. The purposes of this study was to determine if pregnant women with hyperemesis gravidarum were carrying a higher incidence of female fetuses. Materials and Methods: We retrospectively studied ninety-two singleton hyperemesis gravidarum patients admitted to our antepartum institution over tens years period (1980 - 1990). Sixty-six out of ninety-two fetuses could be traced by delivery records or personal contacts through telephone calls. Results: In hyperemesis gravidarum women, forty-four female fetuses and twenty-two male fetuses were identified. The ratio of male to female fetuses was 1:2. In our population group, the ratio of male to female fetuses was 1:0.95. This female fetal predominance in patients with hyperemesis gravidarum was statistically significant ($p < 0.01$). Conclusions: We found that hyperemesis gravidarum women carrying a higher incidence of female fetuses than male fetuses. Whether hyperemesis gravidarum is caused by the female fetal effect on hCG or estradiol concentrations remained to be determined.

108 ESTIMATION OF FETAL RISKS IN ITP PREGNANCIES: A REVIEW OF THE LITERATURE. R.F. Burrows, J.G. Kelton[x], McMaster University, Hamilton, Ontario, Canada.

The management of the pregnant mother with idiopathic thrombocytopenic purpura (ITP) is difficult because it is not possible to predict the fetal platelet count without using invasive tests. The central issue is that the true risk of neonatal thrombocytopenia is not known. To try to obtain an accurate estimate of the actual neonatal risk, we reviewed all English language studies of ITP in pregnancy published in the last decade. Eighty-eight papers described 885 ITP pregnancies producing 893 living infants. To minimize worse case reporting bias, we restricted analysis to those reports that included ≥8 patients and provided all neonatal platelet data. This cohort consisted of 487 ITP pregnancies producing 492 infants. The prevalence of neonatal platelet counts at birth $\leq 50 \times 10^9$/L was 11% (95% CI 8.2-13.8%) and $< 20 \times 10^9$/L was 5.5% (95% CI 3.5-7.5%). Minor morbidity (petechiae, etc.) occurred in 3.3% (95% CI 1.7-4.9%) and major morbidity (intracranial hemorrhage) occurred in 0.4% (95% CI 0.0-1.0%). There was no mortality. Cordocentesis was utilized in 21.7% of this group, vaginal delivery occurred in 28% of those infants with counts $\leq 50 \times 10^9$/L and minor morbidity occurred in 33.3% of those infants $\leq 50 \times 10^9$/L delivered by cesarean section. We conclude: (a) the prevalence at birth of severe neonatal thrombocytopenia ($< 20 \times 10^9$/L) is low; (b) minor and major neonatal morbidities are rare; and (c) interventions are neither mandatory for a good outcome nor ensure one.

109 STILLBIRTHS: WHAT LABORATORY STUDIES ARE HELPFUL?
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There is a national need to standardize the review investigations into the etiologies of fetal demise. LAC-USC Women's Hospital, a large tertiary care referral center, has prospectively collected sociodemographic data, antenatal clinical data, maternal laboratory studies, and when possible, postnatal pathologic evaluation. While necropsy evaluation has recognized benefit, the role of routine laboratory studies remains unclear. This study characterizes the efficacy of common laboratory analyses used in an attempt to define associated causes of stillbirths (SB). METHOD: All SB delivered at LAC-USC during 1990 were identified, sociodemographic, antenatal and delivery data were recorded. On admission, maternal blood type and Rh, CBC, PT, fibrinogen(F), Kleihauer-Betke(KB), VDRL, HbA1C, ANA, and TORCH titers were drawn. RESULTS: 209 SB's and 18,094 total births occurred in 1990. The SB rate was 11.6/1000 in this predominantly Hispanic population. The mean maternal age was 25.8(±6.1). Most women were multiparous and 73% had at least one prenatal visit. Mean number of visits was 5.3(±3.7). Mean gestational age at delivery was 32.7(±6.4).

Table 1: LABORATORY RESULTS

TEST	N(%)	ABNL(%)	TEST	N(%)	ABNL(%)
TORCH	138(66.0)	23(16.6)	KB	82(39.2)	4(4.9)
DRUGS	52(24.8)	8(15.4)	PT	180(85.1)	7(3.9)
ANA	133(63.6)	13(9.7)	F	182(87.1)	4(2.2)
VDRL	108(51.7)	9(8.3)	HbA1C	119(56.9)	1(.8)

CONCLUSION: Contrary to popular belief, markers for hyperglycemia and hematologic abnormality occurred infrequently in mothers with SB. Routine screening of maternal serum for HbA1C, PT, F, and KB should be guided by clinical presentation. Conversely, markers for infection, possible collagen vascular disease and acute drug ingestion may provide diagnostic clues to the etiology of demise in SB. The seroprevalence of ANA and TORCH warrants further evaluation in Hispanic women of reproductive age before widespread screening can be advocated.

110 **EXPECTANT MANAGEMENT OF RESOLVING HYDRAMNIOS.** J. Yu,[X] A. Medearis, R. Paul, University of Southern California, Los Angeles, CA

This study evaluates the efficacy of frequent surveillance measures in patients with hydramnios. Hydramnios was defined as a 4 quadrant vertical pocket amniotic fluid sum of >24 centimeters. Over an 18 month period, 66 patients exhibiting hydramnios entered a tertiary referral center for evaluation. Each patient underwent ultrasonic evaluation of amniotic fluid volume, fetal anatomy, non-stress testing, biophysical profiles, and prenatal visits of increased frequency. Hydramnios resolved spontaneously in 52 (79%) pregnancies. A soft palate cleft was the only anomaly found in these 52 infants. Three infants with suspected anomalies had none found at delivery. The serial ultrasound evaluations, antepartum tests, and weekly visits did not change the management or outcome of these pregnancies. Hydramnios failed to resolve in 14 (21%) pregnancies. This group included five infants with identified antepartum anomalies confirmed at birth and two infants with sonographically undiagnosed anomalies found postpartum. The remaining 7 (50%) infants had normal newborn examinations. In conclusion, it appears that once hydramnios has resolved little is gained with frequent use of antepartum surveillance. Also, half of patients with persistent hydramnios will have a normal newborn examination.

111 M ANTIGEN ISOIMMUNIZATION IN PREGNANCY: A SERIES OF 34 PATIENTS. KE Kern*, TCC Peng and MJ Dinsmoor. Dept of Ob/Gyn, Medical College of Virginia/Virginia Commonwealth of Virginia, Richmond, Virginia.

M antigen is present in 70% of the general population. Although maternal antibody to M antigen (ANTI-M) is listed as a cause of severe hemolytic disease of the newborn (HDN), there are only 5 reports of patients with pregnancy complications attributed to ANTI-M, 4 with no titers reported and one with "low titers". To assess the frequency and outcome of ANTI-M in our pregnant population, we reviewed blood bank records from 12/6/88-5/2/91. 34 patients with ANTI-M were identified, for an approximate incidence of 3.1/1000 deliveries. Titers were performed in 16 patients and ranged from 1:1 to 1:128. Review of maternal and neonatal records reveal that 4/6 (67%) paternal blood types were M-positive. Delta OD450 values were all Zone 1 in the 5 patients who had amniocentesis (Titers 1:4 to 1:128). Mean gestational age and birthweight at delivery were 39.3 wk and 3214 gm (N=33, excluding one Trisomy 18 fetal demise). Review of nursery laboratory records and 25 neonatal charts reveals no cases of HDN, anemia, hyperbilirubinemia or positive direct Coombs. We conclude that although common, ANTI-M rarely causes adverse neonatal outcome.

112 **PREGNANCY IN THE SPINAL CORD INJURED WOMAN.** E. Baker,[X] D. Cardenas,[X] T. Benedetti, University of Washington, Seattle, WA

A retrospective chart review was performed of all patients with traumatic or infectious spinal cord injury (SCI) or spina bifida who delivered at the University of Washington Hospital from 1980-1990. 16 patients with lesions ranging from C4 to S1 levels had 19 pregnancies. There were 10 cases of traumatic SCI, 1 transverse myelitis and 5 spina bifida. Prior medical problems included urinary tract infection (UTI) in 90% and anemia in 63%. The patients' obstetrical care was only remarkable for tocolytic use in 26% and 1 preterm delivery at 34 weeks. Antepartum medical problems included UTI in 70%, pyelonephritis in 26%, and decubitus ulcers in 26% of the patients. 70% of the patients were delivered vaginally and 30% by cesarean section. 5 patients were at risk for autonomic dysreflexia (AD) with lesions at or above T6. 3 patients had 4 episodes of AD treated by spinal anesthesia, delivery or control of preterm labor. Modern medical and obstetrical care allow good outcomes for pregnant spinal cord injured women although significant maternal medical risk exists including urinary tract infections and decubitus ulcers. Autonomic dysreflexia remains a serious complication of labor and delivery occuring in most patients with lesions at or above T6.

113 INITIAL REQUIRED INSULIN DOSE IN GESTATICNAL DIABETICS. J Binderman[X], JC Pastorek II, JM Miller Jr, LSU Medical Center, New Orleans, LA.

Initiating insulin therapy in newly diagnosed gestational diabetics has traditionally been based on formulas using ideal body weight (IBW), correcting for trimester of gestation. We evaluated formulas using both IBW and actual body weight (ABW) to predict the actual amount of insulin (ACTINS) required for glucose control. Charts of 37 insulin-requiring gestational diabetics of a 2 year period were reviewed. Formulas tested were F1: Ins =.7(ABW), F2: Ins =a(ABW), F3 =a(IBW) (a = .6 in 1st trimester, .7 in 2nd, .8 in 3rd). The ABW insulin dose formulas predicted actual dose required. For F2 the mean difference ±SE was -.5±3.5, and for F1 the difference was -4.6±3.5, both not different from zero. The IBW formula differed from actual requirements by -30.5±3.5 (p=.001). Stepwise regression of IBW, ABW, and trimester reaffirmed the value of ABW: Ins = 23.3 + .5ABW (r=.455, p=.005). Using ABW will better predict the actual insulin needed, thus decreasing the time and cost spent in attaining close glucose control.

114 PREGNANCY LOSS AND THROMBOSIS WITH PROTEIN C DEFICIENCY IN PREGNANCY J Trauscht-Van Horn[A], E Capeless, EG. Bovill[A], TR Easterling, B Hermanson[A], Univ of Washington, Seattle & Univ of Vermont College of Medicine

Protein C, a vitamin K dependent plasma protein, inhibits coagulation and promotes fibrinolysis. The deficiency, inherited in an autosomal dominant pattern, increases the risk of venous thrombosis. This study investigates pregnancy loss and thrombotic disease during pregnancy in protein C deficient women.

Materials and Methods: A single New England kindred of 15 protein C deficient cases and 37 related non-deficient controls was studied. An obstetrical history was obtained by phone from all women of child bearing age. Data were analyzed by t test and logistic regression analysis.

Results:

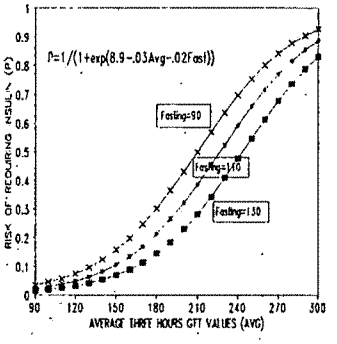
	Cases (15)	Controls (37)
Pregnancy loss(es)	5 (33%)	7(19%)
<12 wks	3	4
>12 wks	2	3
Premature births (<2kg)	0	0
Mean birth weight	3168g	3156g
Birth weight range	2040-4184g	2152-4198g
Thrombosis in pregnancy	5 (33%)	2 (5%)
without anticoagulation	5 (45%)	
Successful pregnancy	15 (100%)	37 (100%)

The odds ratio for thrombosis in pregnancy associated with protein C deficiency was 7.37, (P=.026).

Conclusions: Protein C deficiency is not associated with increased pregnancy loss and is associated with an increased risk of thrombosis in pregnancy.

115 PREDICTING THE NECESSITY FOR INSULIN THERAPY IN THE GESTATIONAL DIABETIC. G.L. Goyert, Y.A.H. Daoud,[X]D.J. Wright,[X]D.B. Schwartz. Dept. Ob/Gyn, Div. MFM, Sinai Hospital, Wayne State University School of Medicine, Detroit, MI.

A minority of gestational diabetics require insulin therapy to maintain fasting glucose < 100 mg/dl and two hour post-prandial glucose < 120 mg/dl. This study was undertaken to model 3-hour oral GTT results to predict the necessity for insulin therapy. Three hour GTT results of 317 patients referred for management of gestational diabetes between 1988-91 were analyzed via step-wise logistic regression. Independent variables entered into the analysis included the four values of the 3 hour GTT individually, the difference between each post-ingestion value and the fasting value, and the mean of the values obtained following ingestion of the glucola. Sixty nine patients required insulin therapy. The average of the three post-ingestion values (improvement χ^2=59.3) and the fasting value (improvement χ^2=11.7) were the two most significant predictors for necessity of insulin therapy. These data facilitate counselling the gestational diabetic and may influence the timing of insulin therapy initiation, especially "prophylactic" insulin regimens.



116 IMPROVED PERINATAL OUTCOME AFTER ENHANCED PRENATAL CARE FOR OPIATE ADDICTS.
G Chang*, KM Carroll*, HM Behr*, NJ DeGennaro*, TR Kosten*, RS Schottenfeld*, and RR Viscarello.

Chemical dependency during pregnancy is frequently correlated with poor prenatal care and adverse perinatal outcomes. Nearly 75% of the estimated 300,000 female narcotic addicts in the United States are of childbearing age. Pregnant opiate addicts have a six-fold increase in maternal complications including IUGR, premature labor, third-trimester bleeding, and malpresentation. Newborn complications include neonatal withdrawal syndrome, failure-to-thrive, SIDS, and neurobehavioral problems. In order to improve perinatal outcome for opiate-addicted women, we developed an enhanced program which combines on-site prenatal care, methadone maintenance, supervised childcare, and relapse-prevention strategies. As part of our pilot phase, 12 of 23 pregnant women currently on methadone maintenance agreed to participate in the enhanced program. The 11 patients who declined were followed in a traditional prenatal care setting. Both groups had similar demographics including age, years of education, marital status, racial breakdown, parity, and daily methadone dose. Six of the patients in the enhanced group have delivered, 3 elected TOP, and 3 remain undelivered; while in the routine group, 6 have delivered, 1 miscarried, 2 underwent TOP, and 2 are lost to follow-up. Despite a greater mean number of urine toxicology screens (43 vs. 15), subjects in the enhanced program demonstrated a lower percentage of positive results (59% vs. 76%). The enhanced group had significantly more prenatal visits (8.8 vs. 2.7), longer gestations (38.2 wks vs. 35.7 wks), and larger infants (2943g vs. 2280g). Preliminary results suggest improved outcomes in opiate-dependent patients receiving enhanced as compared to routine prenatal care. Such a therapeutic approach also provides the addicted woman with the structural framework to organize her own life and thereby improves parenting skills.

117 MANAGEMENT OF PREGNANCIES IN DIABETIC WOMEN: CAN WE DO BETTER? U. Lang*, G. Braems*, K. E. Clark*, W. Kuenzel, Frauenklinik der Justus -Liebig - Universitaet, Giessen, Germany and Univ. Cincinnati, Cincinnati, Ohio.

Modern management of pregnancies in diabetic women (PD) in specialized centers has improved the rates of perinatal mortality and morbidity to approach those in the non-diabetic population. To determine whether these rates of improvement are valid for a large population, data from the Hessische Perinatalstudie, a computerized system of collecting information on obstetrical care in the State of Hesse, Germany (Pop. 5.6 million) were used to compare 446 PD (0.4% of 111836 pregnancies recorded from 1982-86) to 707 PD (0.5% of 145025 pregnancies recorded from 1987-89). Patient histories, pregnancy risks, birth risks, fetal outcome and maternal well-being were evaluated in the specific setting of widely decentralized obstetric care. Perinatal mortality in infants of diabetic mothers (IDM) dropped from 4.9% to 2.4%, but still remained substantially higher than in the non-diabetic population (0.7% and 0.6% respectively). Two thirds of fetal loss occurred before birth. Although neonatal morbidity decreased, the percentage of anomalies and the percentage of macrosomic IDM remained unchanged. These data show, that changes in management and awareness of the PD's problems improved the perinatal outcome, but not to the extent possible in specialized facilities. Preconceptional counseling and therapy (anomalies), glycemic control during pregnancy (macrosomia), fetal monitoring (intrauterine deaths) and experienced care have to be further stressed.

118 **A COMPARISON OF SONOGRAPHIC INDICES IN THE EARLY PRENATAL DIAGNOSIS OF HYDROCEPHALUS:** I. Goldstein[X], Reece EA, Pilu G, Hobbins JC, Departments of Obstetrics and Gynecology at Rambam Medical Center, Israel, Temple University, PA, The University of Bologna, Italy and Yale University, CT

Hydrocephalus is a condition which affects primarily the lateral cerebral ventricles with variable involvement of the other ventricles. Since the incidence of chromosomal anomalies and other structural malformations is increased with hydrocephalus, prenatal diagnosis is desirable. Although various ventricular biometric parameters have been used, the relative accuracy of each remains undetermined. In this study, 51 pregnancies were examined prospectively until frank hydrocephalus developed. Sonographic indices which were applied during early pregnancy (16-24 weeks) were compared to assess the relative accuracy of the various measurements of the lateral ventricle (Lv) and to aid in the early prenatal diagnosis of hydrocephalus. The diagnostic accuracy of each parameter was as follows:

Ventricular Dimensions		Diagnostic Accuracy
Lateral ventric width/ Hemispheric width (LVW/HD)	=	86%
Atrial width/Cerebroatrial distance (AW/CAD) or atrial with <1cm	=	94.1%
Cerebrosprontal distance/ Hemispheric width (CFD/HW)	=	76.3%

CONCLUSIONS: These data demonstrate that abnormal ventricular biometry is present even in early ventriculomegaly. However, the atrial dimension seems to be the most accurate diagnostic parameter and a useful tool for the early prenatal diagnosis of hydrocephalus.

119 **ARDS IN THE OBSTETRIC PATIENT – 18 RECENT CASES**

V. Catanzarite, D Wilms*, J G Quirk, L Cousins, J Schneider
Maternal-Fetal Medicine, Sharp Memorial Hospital, San Diego, CA and UAMS, Little Rock, Arkansas.

Adult respiratory distress syndrome is emerging as a major cause of maternal mortality. We have treated 18 cases over the past four years; all but two patients were delivered at or transferred to, UAMS or Sharp. Causes were obstetric in seven patients (preeclampsia 4, acute fatty liver 2, amnionitis 1), and nonobstetric in 11 (aspiration 2, malignancy 2, viral infection 3, bacterial sepsis 3, and status epilepticus with multiple organ failure 1).

Two mothers in extremis at initial consultation at outside hospitals were hemodynamically unstable and could not be transported. Both died. Of the 16 managed at Sharp or UAMS, there were 5 maternal deaths - two patients with malignancy and sepsis, 2 with HELLP syndrome, sepsis and multiple organ system failure, and one with aspiration with motor vehicle trauma. One survivor required 28 days of extracorporeal support (ECCOR) and a total of 61 days intubation; others required 3 to 26 days of intubation.

Two previable fetuses died with the mother. There were two additional fetal deaths prior to perinatal consultation, for a perinatal mortality rate of 333 per 1000. Two very premature infants died after delivery.

From our experience:

1) Excepting two patients with malignancy, four of five maternal deaths occurred in patients with multiple organ system failure; only two patients with ARDS and failure of two or more other organ systems survived.
2) All of our patients with antepartum ARDS either miscarried, delivered, or had a demise prior to extubation.
3) Seven of eight patients requiring prolonged intensive support survived, all without residua.

120 **VERY TIGHT GLYCEMIC CONTROL IN GESTATIONAL DIABETES: A BENEFIT OR A DETRIMENT?** Michele K. Presinger, M.D.,[X] Sophia Scarpelli, Michael Y. Divon, M.D., from the Departments of OB/GYN, Albert Einstein College of Medicine, Bronx, N.Y. and Temple University School of Medicine, Philadelphia, PA

Gestational diabetes mellitus (GDM) is classically associated with fetal macrosomia; however, tight glucose control may result in decreased substrate availability. Purpose: to evaluate the incidence of intrauterine growth retardation (birthweight ≤10th percentile) in GDM and its association with maternal mean blood glucose (MBG). 661 consecutive GDMs were studied: 293 were diet controlled (GDM-D), 368 received diet and insulin therapy (GDM-D&I), and 468 low-risk, non-diabetics served as controls. IUGR was detected in 17.4% of GDM-D&I which was significantly elevated over the 7.2% detected in GDM-D and 5.8% detected in controls (P <0.0001). Of the 85 GDMs with IUGR, 41 consecutive patients (31 GDM-D&I, and 10 GDM-D) had multiple daily glucose determinations. Similar glucose data were available for 82 GDMs matched for mode of therapy and gestational age at delivery who delivered appropriate for gestational age sized infants (AGA).

The odds ratios for IUGR in GDM-D&I were 3.5 (95% C.I.=1.3-9.8) with a MBG of 90-100 mg/dl and 5.7 (95% C.I.=1.2-26.3) with a MBG <90 mg/dl. The incidence of C/S for fetal distress and of NICU admissions was significantly higher in GDM-D&I with IUGR (p <0.05). Hypertension, smoking, maternal body mass index and maternal weight gain were not significantly different between IUGRs and their controls. Conclusion: the incidence of IUGR is significantly increased in GDM-D&I, and the likelihood of IUGR increases as the MBG decreases.

Table 1-Incidence of IUGR

Diag/Rx	Incidence-IUGR
GDM-D&I	64/368(17.4%)*
GDM-D	21/293(7.2%
Nondiab	28/486(5.8%) *

*p<0.000001

Table 2-MBG & IUGR

	(90-100mg/dl	<90 mg/dl)
odds ratio	3.58%	5.7%
95%conf int	1.3-9.8%	1.2-26.3%
p value	0.009	0.02

121 **PREDICTION OF THE NEED FOR INSULIN THERAPY IN GESTATIONAL DIABETES:** N Vohra, G Berkowitz, R Lapinksi*, L Lynch, CJ Lockwood. Mt Sinai School of Medicine, New York, NY

Insulin therapy in gestational diabetes mellitus (GDM) is often unduly delayed and fails to prevent diabetic fetopathy. This study sought to identify demographic and laboratory indices associated with the need for insulin therapy. Employing an obstetric database 913 GDM patients were identified by an abnormal glucose tolerance test (GTT) between 1986-1990. Despite dietary intervention, 220 patients (24%) required insulin therapy due to fasting whole blood glucose levels > 95 mg/dl or post-prandial levels > 120 mg/dl. Significant differences between patients requiring insulin (IRx) and those responding to diet alone (DRx) included:

Variable		IRx mean (SD)	DRx mean (SD)	t-test/X^2
Pre-pregnancy Wt.	(lbs.)	161 (44)	141 (35)	0.0001
Body Mass Index	(kg/m^2)	28.8 (7.1)	25.4 (6.0)	0.0001
1 hr. glucola	(mg/dl)	165 (29)	156 (27)	0.0004
Fasting GTT	(mg/dl)	101 (20)	93 (14)	0.0001
1 hr. GTT	(mg/dl)	207 (31)	196 (24)	0.0001
2 hr. GTT	(mg/dl)	179 (36)	169 (27)	0.0004
3 hr. GTT	(mg/dl)	136 (41)	129 (32)	0.02
Gest. Age at GTT	(wks)	24 (7)	28 (6)	0.0001
Non-white race		81.8%	53.4%	0.0001
Family History DM		39.5%	23.3%	0.0001
Prior History GDM		19.5%	9.0%	0.0001
Prior insulin use		8.1%	1.6%	0.0001

Logistic regression analysis confirmed that the maternal race, family history of DM, prior insulin use, glucola, fasting and 1 hour GTT values were independent predictors of future insulin use. Therefore a cumulative score for each patient was assigned by multiplying the presence or absence, or value of each risk factor by the appropriate beta and summing. The optimal cut off score, for prediction of insulin use, as assessed by the Receiver-Operator Analysis displayed a sensitivity of 70% and specificity of 70%. SUMMARY: The need for insulin therapy in GDM can be predicted by using a combination of maternal characteristics and laboratory values at the time of initial diagnosis.

122 POLYCISTIC OVARY (PCO): A RISK FACTOR FOR GESTATIONAL DIABETES (GD)? A. Caruso [X], N. Di Simone [X], A. Lanzone [X], S. De Carolis [X], S. Mancuso [X]. Dept Ob/Gyn, Catholic University, Rome, Italy.

We found that patients with PCO had a greater secretion of Insulin (I) than controls and about 60% of them showed an hyperinsulinemic (HI) response to OGTT (Hum. Repr.,1990). Eight pts became pregnant within 6 months following the evaluation of metabolic status (2 obese, 2 HI obese, 2 HI, 2 not HI not obese patients). They were tested at 28-30 wks of gestation by OGTT. All these pts had an increase of I secretion from 100 to 200%. The integrated secretory area of insulin (ISA I), calculated by trapezoidal rule, of PCO pts was greater than that found in 10 healthy women ($p<0.01$) or in 10 non PCO pts with GD ($p<0.01$) tested at the same gestational age and with the same body mass index. GD group had also a decreased ISA I in comparison with controls ($p< 0.05$). Furthermore the four HI PCO pts developed GD ($n=2$) or IGGT ($n=2$). Their ISA I was 50% greater than controls; the other non HI PCO pts showed an ISA I after OGTT at the highest values of control group. These data suggest that PCO pts may be considered a specific subgroup of subjects, who may develop a derangement of the glycemic control in spite of their remarkable increase of I secretion during pregnancy. Additional studies need to the knowledge and clinical management of both normal and hyperinsulinemic GD and IGGT in PCO pregnant patients.

123 MATERNAL COCAINE AND MARIJUANA USE DETERMINED BY ANALYSIS OF AMNIOTIC FLUID AND CORD BLOOD AT DELIVERY. C Lowery, C Lawler[X], JL Valentine[X], Departments of Obstetrics & Gynecology and Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas

Nineteen women presenting for delivery were suspected to be at high risk for substance abuse. Maternal urine and blood, amniotic fluid (AF), and cord blood were collected at delivery. Urine was screened for benzodiazepine, marijuana and cocaine metabolites, opiates and amphetamines using the enzyme multiplied immunoassay technique (EMIT). Only marijuana and cocaine metabolites were found. Three patients had a positive urine screen for cocaine metabolite, two were positive for cocaine and marijuana metabolites, and two were positive for marijuana metabolite only (36% positive for substance abuse). Amniotic fluid testing for these metabolites using EMIT was negative. Maternal blood and urine, AF, and cord blood were analyzed using gas chromatography/mass spectrometry (gc/ms). Cocaine and its major metabolite, benzoylecgonine were found in all body fluids; 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, a major metabolite of the psychomimetic agent found in marijuana, was identified in both urine and AF. The relationship between levels found in the four body fluids was variable and is most likely due to differences in metabolism and excretion of the drugs following dosing intervals (which could not be determined). These results represent the first report of cocaine and marijuana metabolites in human AF as determined by gc/ms. Conclusion: Amniotic fluid may represent a potential pharmacokinetic compartment to assess fetal exposure.

124 AN ANONYMOUS COMPARISON OF SUBSTANCE ABUSE BETWEEN CLINIC PATIENTS AND LABOR & DELIVERY PATIENTS IN A RURAL SOUTHERN STATE. CL Lowery, C. Crone,[X] JM Benanti, R Kirby,[X] J Valentine,[X] Departments of OB/GYN and Pediatrics, University of Arkansas for Medical Sciences. Little Rock, Arkansas.

Maternal urine was collected during a 30 day period from all patients seen in prenatal clinic (N=386) or on the Labor & Delivery (L&D) Unit (N=227). Urine samples were analyzed for benzodiazepine, marijuana, cocaine metabolites, opiates and amphetamines using enzyme multiplied immunoassay technique (EMIT), and all positive results were confirmed with gas chromatography/mass spectrometry (gc/ms). Prevalence rates for marijuana and cocaine were 6.7% and 2.2%, respectively, among in-patients, and 7.6% and 0.8%, respectively, among out-patients. No difference in the prevalence rate for marijuana or cocaine was found between in- and out-patients according to race, maternal age, gestational age, or payment source. Among out-patients, black women were more likely than white women to use cocaine; in general, women $\geq$ 37 weeks were more likely to be positive for cocaine ($p<.005$). Among in-patients, lower gestational age was associated with marijuana use ($p<.05$), and lack of prenatal care was associated with cocaine use ($p<.005$). In this study, a lower positive rate was found during urine drug screening than has been reported elsewhere from socioeconomically similar populations. This lower rate presumably reflects a lower incidence of substance abuse at a teaching hospital in a rural southern state.

125 ANONYMOUS DRUG SCREENING OF PRENATAL PATIENTS BY PAIRED URINE COLLECTION AND DRUG HISTORY. CL Lowery, C Crone,[x] R Kirby,[x] J Valentine.[x] Departments of OB/GYN and Pediatrics, the University of Arkansas for Medical Sciences. Little Rock, Arkansas.

Concerns over legal ramifications and confidentiality imposed by universal urine drug screening prompted a study to compare urine screening to a nurse-administered drug screening form. During a 30 day period all patients (N=386) receiving prenatal care in the outpatient clinics were screened by both. Urine (collected anonymously) was analyzed by enzyme multiplied immunoassay technique (EMIT) for benzodiazepine, marijuana, cocaine, opiates, and amphetamines. Smoking correlated most with urine results (sensitivity: 83.3%, positive predictive value: 20.7%). Only 23.3% of women testing positive admitted current use; of those admitting to current use, only 50% tested positive. Since urine screening alone will miss users, nurse-administered questionnaires offer a reasonable alternative to universal urine screening.

	Sensit. %	PPV %	Number Pos. (%)
Current Use	23.3	50.0	044 (03.6)
Past Use	56.7	20.0	100 (25.9)
Smoking	83.3	20.7	121 (32.8)

126 THE EFFECT OF CONTINUOUS AND PULSE COCAINE EXPOSURE ON ENDOTHELIAL CELLS OF HUMAN UMBILICAL CORD

D.S. Mastrogiannis[x], W.F. O'Brien. Depts. of OB/GYN at Temple University School of Medicine, Phila., PA, and University of South Florida College of Medicine, Tampa, FL.

There is an apparent association between cocaine abuse and adverse perinatal outcome. It has been postulated that some of the adverse effects of cocaine usage in pregnancy may be due to a disturbance of the endothelium with a secondary reduction in the release of vasodilatory prostaglandins. We have recently shown (SGI, 1991, Abstract #3) that cocaine decreases prostacyclin production when pharmacological doses of cocaine are added in human umbilical vein endothelial cell (HUVEC) culture. In the present study, we examined the effect of low dose cocaine concentrations and for variable time periods in a continuous and pulsed in vitro model. Confluent cultures of human umbilical cord endothelial cells, in 24 well plates were incubated with culture medium containing 0, 250, 500 ng/ml of cocaine for 6 days, 24 hr. a day in the continuous experiment and 2 hr. a day in the pulsed one. The medium was changed daily and was assayed for 6 keto-$PGF_{1\alpha}$ the stable metabolite of prostacylin (PGI_2) by RIA.

RESULTS: 6 keto $PGF_{1\alpha}$ (µg/.1ml)

	PULSE			P	CONTINUOUS			P
Cocaine	0	250	500		0	250	500	
Day 1	448	431	410	NS	619	535	476	NS
Day 3	285	283	257	NS	478	508	546	NS
Day 4	399	379	327	NS	449	464	388	NS
Day 5	313	322	294	NS	304	381	395	NS
Day 6	408	446	294	NS	347	361	403	NS

CONCLUSIONS: Cocaine in low doses and variable time periods did not affect the production of prostacyclin when incubated with HUVEC in our in vitro model. These data suggest that PGI_2-mediated adverse perinatal complications might require higher doses and thus greater prenatal surveillance is warranted.

127 PRENATAL COMPLICATIONS IN INSULIN-DEPENDENT DIABETIC PREGNANCIES. B. Rosenn, M. Miodovnik, J. Khoury,[x] T.A. Siddiqi, Dept. Ob/Gyn, Univ. Cincinnati Med. Ctr., Cincinnati, OH

Insulin-dependent diabetic women are considered at high risk for prenatal complications of pregnancy; however, sound descriptive data are scarce and most suffer methodologic drawbacks. In order to establish the actual rates of these complications in our diabetic population and to improve our ability to provide patients with prenatal counseling, we retrospectively studied 254 insulin-dependent diabetic women (White classes B-RT) enrolled in our multidisciplinary program of diabetes in pregnancy prior to 20 weeks' gestation and followed every 1-2 weeks throughout pregnancy. Goals of glycemic control were fasting blood glucose < 100 mg/dl and 90 minutes postprandial blood glucose < 140 mg/dl. A control group of 508 non-diabetic women were randomly selected from the obstetric population enrolled in the hospital's prenatal clinics prior to 20 weeks' gestation, and matched to the diabetic group (ratio 2:1) by age, race, and parity. Diabetic patients had significantly higher rates of preeclampsia (34% vs. 7.7%; p<.001), polyhydramnios (31% vs. 0.6%; p<.001), pyelonephritis (4.1% vs. 1.4%; p<.03), meconium stained amniotic fluid (11.4% vs. 4.5%; p<.001), and spontaneous preterm delivery (12.4% vs. 7.3%; p<.05). No difference was found in the rate of premature rupture of membranes. Preeclampsia and polyhydramnios were associated with microvascular disease and higher mid-trimester glycohemoglobin concentrations. We conclude that the rate of prenatal complications of pregnancy is indeed increased in insulin-dependent diabetic pregnancies. We speculate that the factors predisposing to such complications are already established by mid-pregnancy and that early glycemic control may be required to decrease their frequency.

128 RISK FACTORS FOR INTRAUTERINE FETAL DEATH

RL Copper[x], RL Goldenberg, MD DuBard[x], RO Davis. The University of Alabama at Birmingham, Birmingham, Alabama.

Risk factors and medical origins of 403 stillbirths which occurred in 5 perinatal centers from 1982-86 were studied. The population included 34,351 births of women screened as part of the March of Dimes Multicenter Preterm Birth Prevention Trial. Stillbirth (SB:Apgar=0 at 1 and 5 min at ≥ 20 wks GA) occurred in 1.2% of all births. 51% of SBs occurred before 28 wks and only 18% occurred at term. Blacks had an increased risk of SB compared to whites (RR 1.5, p<.001) and Hispanics had a reduced risk (RR .7, p<.05). Work, parity, and age < 17 were not associated with SB, but age >35 (RR 2.2, p<.001) and single marital status (RR 1.7, p<.001) were associated. Thin women did not have increased risk of SB but women >85kg had a RR of 2.4 (p<.001). Both prior spontaneous (RR 1.95, p<.001) and indicated PTDs (RR 3.2, p<.001) were associated with SB. Preeclampsia resulted in no increased risk, yet the RR of SB related to chronic hypertension was 2.1 (p<.001). Class A Diabetes (DM) had no increased risk while the RR of SB in Class B-R DM was 2.1 (p=0.05). Conditions resulting in the highest RR of SB were hemoglobinopathies, (7.2, p<.001), Rh sensitization (4.4 p <0.01), and abruption (14.9, p<.001). These data may assist in identifying a fetus at risk for SB and provide data upon which to base studies aimed at reducing fetal death.

129 **THE INCIDENCE AND NATURAL HISTORY OF ASYMPTOMATIC CHOLELITHIASIS IN PREGNANT PATIENTS.** J. Williams III, R. Willis-Hassan[X], D. Aiken-Hunting[X], B. Caro[X], J.S. Greenspoon, The Prenatal Diagnostic Center of So. CA & Dept. of Ob/Gyn, Cedars-Sinai Med. Ctr, Beverly Hills & Los Angeles, CA

We prospectively evaluated 186 patients for cholethiasis to determine the incidence and natural history of cholelithiasis in women diagnosed during pregnancy. Symptomatic gallstones diagnosed during pregnancy are associated with a 15% rate of pregnancy complications. We hypothesized that asymptomatic gallstones infrequently become symptomatic, and are seldom associated with pregnancy or medical complications. A realtime ultrasound examination of the biliary tract was performed at the time of an indicated second trimester fetal evaluation. Patients included in the study were referred for genetic or obstetrical indications. No patient was referred for evaluation of biliary tract disease. The mean (SD) maternal age was 35.7 (4.5) years. The mean maternal weight was 68.2 (11.8) kilograms. The mean maternal height 1.64 (0.06) meters. The mean body mass index (BMI) was 25.3 (4.2). The gestational age at the time of the examination was 16 to 24 weeks. An adequate examination of the biliary tract was achieved in 176 (95%) of patients. An adequate exam was obtained as frequently in overweight patients with a BMI$\geq$27 as in patients with a BMI<27. Although 5 of 55 (9%) patients with BMI$\geq$27 had inadequate examinations compared to 5 of 131 (3%) non-obese patients (BMI<27), the difference was not statistically significant. Four of 176 (2%) evaluable patients had cholelithiasis. In retrospect, symptoms attributable to cholelithiasis were elicited from one of the 4 patients with cholelithiasis. None of the 4 patients has required therapy, although pregnancies are ongoing. Further enrollment of consecutive patients is in progress to test the hypothesis.

130 SEXUAL ASSAULT IN PREGNANCY: A SURVEY OF 2404 WOMEN. AJ Satin,[X] J. Paicurich,[X] S. Millman,[X] G.D. Wendel, Dept. Ob/Gyn, U. Texas Southwestern Med Center., Dallas, TX

A women is sexually assaulted every 6 minutes in the U.S., however little data exists on the prevalence of and effect of rape on an obstetric population. In a previous retrospective study of assault victims we estimated an incidence of rape of 0.55/1000 pregnancies. The purpose of this investigation was to determine the prevalence of sexual assault in urban gravidas, characterize pregnancy complications and report pregnancy outcome of assault victims. Women (n=2404) were interviewed within 48 hours of delivery regarding forced sexual contact before or during the current pregnancy. Reported pregnancy complications were confirmed by review of obstetric, medical & police reports. A history of sexual assault was elicited in 50:1000 (n=120) women. The incidence of assault during the current gestation was 2:1000 (n=5). Rape victims were more likely to be white (43v.16%, P<.001), employed (28v.19%, P<.02), high school graduates (23v.14%, P<.005) and separated or divorced (13v.5%, P<.001), compared to non-victims. Rape victims had a higher incidence of STD's (9v.4%, P<.01), UTI's/vaginitis (32v.21%, P=.02), drug use (9v.2%, P<.001), and multiple hospitalizations (15v.8%, p<.01) during pregnancy. There were no differences in neonatal outcome reflected by umbilical artery acidosis, EGA at delivery, or birthweight. Thus in our gravid population a history of sexual assault is common. Rape in pregnancy occurs four times more often than previously estimated. Assault victims have more frequent pregnancy complications, but achieve normal pregnancy outcome.

131 **3 HOUR GLUCOSE TOLERANCE TEST (GTT) RESULTS ARE UNRELATED TO OUTCOME IN A SELECTIVELY SCREENED POPULATION.** Michael J. Lucas, Thomas W. Lowe, Lisa Bowe,[X] Donald McIntire,[X] Dept. Ob/Gyn, Univ. Texas Southwestern Medical Center, Dallas, TX

Selective GTT testing of a large cohort of antepartum clinic patients resulted in the identification of 126 class A_1 diabetics and 139 gravidas with normal glucose tolerance based on a 100 gm, 3 hr GTT. These two groups were compared to evaluate the relationship of glucose intolerance to perinatal outcome. The Class A_1 diabetics received dietary counseling and had fasting blood sugars checked at the time of antepartum clinic visits, but otherwise received routine obstetric care. There was no significant difference between groups in average birthweight, EGA at delivery, stillbirths (one each), cesarean section delivery rate, meconium staining of fluid, cord pH (or % < 7.2) or Apgar scores. There were very few small for gestational age (SGA) neonates (2 in each group), and although there were 36% large for gestational age (LGA) neonates in the diabetics, this was not statistically different from the 31% LGA delivered in the normal GTT group. There was no significant difference in the 3 hr GTT profiles between mothers of LGA neonates and the others within each group. There was a 5% incidence of shoulder dystocia in the LGA neonates, and half of these had evidence of injury. There was no apparent relationship between neonatal size and route of delivery. Maternal weight, on the other hand, was significantly higher in cesarean deliveries and in LGA mothers, but was not significantly different between the GTT result groups. The high incidence of LGA neonates observed in both GTT groups suggest that either the abnormal 50 gm result or the indications for screening selected for this outcome. It should be noted that maternal obesity was not an indication for screening. Prior delivery of a macrosomic infant was the indication in 22% of mothers screened yet cortributed 44% of the LGA neonates. While not surprising that factors other than maternal glucose tolerance affect fetal size, the lack of association with GTT results indicates that targeting glycemic control in this population would not significantly reduce the incidence of LGA outcome.

132 CHARACTERISTICS AND OUTCOMES ACCORDING TO DIAGNOSTIC CRITERIA FOR GESTATIONAL DIABETES. G. Berkowitz[X], R. Lapinski[X], M. Alvarez, C. Lockwood, R. Berkowitz. Mount Sinai School of Medicine, New York, NY

The criteria endorsed by the National Diabetes Data Group (NDDG) for determining abnormal plasma glucose values were converted from O'Sullivan's original values based on whole blood without taking into account differences between whole blood and plasma values. Carpenter and Coustan (1) have calculated lower thresholds based on an adjustment for this difference. Our institution, which routinely screens all patients on the clinic service for diabetes, has adopted the lower thresholds. The aim of this study was to assess whether maternal characteristics and neonatal outcomes differ betwwen those who meet the NDDG criteria and those who only meet the criteria of Carpenter and Coustan. A total of 233 patients were diagnosed with gestational diabetes (GDM) based on the NDDG criteria and an additional 121 patients met the lower threshold criteria. The distributions of maternal age, race/ethnicity, body mass index, weekly maternal weight gain, a family history of diabetes, and a history of stillbirths or spontaneous abortions were very similar for the two groups. However, insulin use was more common in the NDDG group (p=0.046) and there was a suggestion that a prior history of GDM was more frequent in this group (p=0.06). With regard to neonatal complications, there were no significant differences between the two groups although a birth weight >.90th percentile for gestational age tended to be more common in the NDDG group (p=0.06). SUMMARY: Although there was some suggestion that the patients fulfilling the NDDG criteria exhibit more sequelae of glucose intolerance than those who only meet the criteria proposed by Carpenter and Coustan, no clear distinctions were observed in terms of maternal characteristics or adverse neonatal outcomes between the two groups.

(1) Carpenter M, Coustan D. Am J Obstet Gynecol 144:768, 1982.

133 *THE ROLE OF AMNIOTIC FLUID DISTRIBUTION PREDICTING PERINATAL OUTCOME IN PATIENTS WITH RUPTURED MEMBRANES.* *TD Myles, HT Strassner, Rush Medical Center, Chicago, IL*

In our previous study amniotic fluid index (AFI) distribution was predictive of perinatal outcome in patients presenting for delivery with intact membranes. This study evaluated whether similar predictive values existed for patients presenting with spontaneous rupture of membranes. An AFI was performed on 84 patients. Those with >50% of the AFI in the upper quadrants were placed in the upper greater group (UG). All others were classified lower greater (LG). The LG AND UG groups were compared in relation to meconium staining (MEC), 1 or 5 minute Apgars <7, persistant variable decelerations (VD), late decelerations (LD), umbilical artery or vein pH <7.20, NICU admission, and cesarean sections for fetal distress (CSFD). These findings were similar to those in our earlier study. Patients in the UG group had a higher incidence of MEC (29.8% vs 2.7%, p <.002), 1 minute Apgar <7 (19.1% vs 2.7%, p <.021), VD (61.7% vs 24.3%, p<.001), LD (23.4% vs 0%, p<.002), and CSFD (12.8 vs 0%, p<.024). There were no significant differences between groups with regard to overall AFI.

134 **THE FETAL HEART RATE CHANGES DURING THE FIRST STAGE OF LABOR IN LOW-RISK PREGNANCIES.** N. Field,* A. Samueloff,* M. Berkus, E. Xenakis,* L. Ridgway, O. Langer. Dept. OB/GYN, Univ. of Texas Health Science Center, San Antonio, TX.

Few studies document the changes in the characteristics of fetal heart rate (FHR) during the first stage of labor. Therefore we investigated the natural history of the FHR tracing throughout the first stage of labor in normal, uncomplicated pregnancies. Five hundred and twenty-nine women who met all of the following admission criteria were included in the study: gestational age >36 and <43 weeks, vertex presentation, vaginal delivery, birthweight >2500 gm, and good neonatal outcome. Infants with a good outcome went home in 2-5 days with no NICU admissions. Cases with either twins, malformations, or maternal disease (diabetes, hypertensive complications, antepartum bleeding, chorioamnionitis, materna substance abuse, suspected growth retardation) were excluded from analysis. The entire first stage of labor was documented in all study cases. Two 30-min segments of fetal heart tracing, one immediately upon admission and the second, one hour prior to complete dilation, were evaluated. The following parameters of the tracing were compared between the two different time frames:

FHR Parameter	FHR at admission	FHR 1 h prior to complete dilation	p value
Baseline (bpm)	138±9.2	138±9.9	NS
No. oscillations/min	6.8±2.1	7.1±2.6	.02
Total no. accel./min	4.5±3.6	2.9±3.1	<.0001
No. accel. >15 beats	3.7±3.8	2.3±3.0	<.0001
Highest accel. (beats)	21±14	17±16.7	<.0001
No. contractions/min	8.2±3.9	11.6±3.6	<.0001
Total no. decel./min	0.5±1.6	2.8±3.7	<.0001
No. variable decel./min	0.3±1.2	2.6±3.6	<.0001

Additionally, 19.2% of cases with a normal tracing (after Krebs) on admission ended the first stage of labor with abnormal scores. Furthermore, 56.5% of cases with abnormal tracings at entry completed the first stage with normal scores. In conclusion, our data show some deterioration of the FHR pattern during the first stage of labor in a population of low-risk pregnancies with good neonatal outcomes.

135 **PROLONGED BRADYCARDIA: HOW LONG IS LONG ENOUGH?** N. Field,* A. Samueloff,* M. Berkus, E. Xenakis,* M. McFarland,* O. Langer. Dept. of Ob/Gyn, UTHSC, San Antonio, TX.

Disagreement exists regarding the significance of prolonged bradycardia occurring in the second stage of labor. We hypothesized that the duration of the prolonged bradycardia in the second stage is not associated with adverse perinatal outcome in the absence of clinically-evident obstetrical emergency, e.g., placental abruption or uterine rupture. In an ongoing study, we analyzed the fetal heart rate tracings in the entire second stage of labor for 1,500 consecutive deliveries. Prolonged bradycardia was defined as either a fetal heart rate ≤90 beats per minute or a drop in the previously established baseline of ≥30 beats lasting for ≥2.5 minutes. Perinatal outcome was assessed by 1 and 5 minute Apgar scores and umbilical cord pH at delivery. Additionally, infants who went home within 2.5 days with no NICU admissions were defined as having a good outcome. Ninety-four cases of prolonged bradycardia in the second stage of labor were identified, representing an incidence of 6.5% of all cases analyzed. The length of the bradycardia ranged from 2.5 to 20 minutes, with a mean duration of 5.6±1.9 minutes. The mean drop in fetal heart rate from baseline was 53.4±36 beats. Forty six (63.0%) of the patients had a normal spontaneous vaginal delivery and twenty-six (35.6%) were delivered by vacuum extraction or forceps; only one patient (1.3%) underwent cesarean section.

	Length of Prolonged Bradycardia (Minutes)			
Outcome Variable	*<3 min (n=28)*	*3-5 min (n=29)*	*5-7 min (n=19)*	*>7 min (n=18)*
pH >7.20	75.0%	86.4%	68.8%	71.4%
1 min Apgar >7	81.0%	95.5%	87.5%	92.9%
5 min Apgar >7	100.0%	100.0%	93.8%	100.0%
Good outcome	100.0%	82.6%	78.9%	91.7%

The study further revealed: 1) the absolute length of the second stage of labor did not seem to influence outcome in the presence of prolonged bradycardia, and 2) the mode of delivery did not affect the fetal outcome. Thus, our data suggest that in the face of a prolonged bradycardia in the second stage of labor, expeditious delivery is not a necessary intervention to guarantee a good fetal outcome.

136 CORD CREATINE KINASE BB ISOENZYME CORRELATES WITH FETAL METABOLIC ACIDOSIS. R. Soper, U. Verma, N. Tejani, NY Med., Coll., Valhalla, N Y.

Background Conventional methods of peripartum fetal assessment have poor sensitivity and predictive value for immediate and long term fetal outcome. Tissue damage derived enzymes may be indicators of fetal injury. Creatine Kinase (CK) an energy transfer enzyme, exists in three isoenzyme forms. The BB form is derived predominantly from neural tissue. **Object** Correlation of cord CKBB with fetal metabolic acidosis in the term fetus. **Method** 139 consecutive deliveries of ≥35 weeks gestation were studied. Patients were divided into 2 groups based on the umbilical artery base deficit (BD), acidotic BD≤-10mMol/L and non-acidotic BD> -10 mMol/L. Total CK and CKBB in the umbilical artery and vein were correlated with acidosis. **Results** umbilical artery and vein CKBB correlated significantly with metabolic acidosis. **(TABLE)**

		BD≤ -10mM/L (#21)	BD> -10mM/L (#118)	P
Artery	CKBB U/L +/-SEM	62.1 +/-35	19 +/-2.7	0.02
Vein		26.7 +/-5.4	18.9 +/2.4	0.03

Conclusions CKBB correlates with fetal metabolic acidosis and may be a more specific predictor of neurological injury than other outcome markers.

137 INTRAPARTUM FETAL HEART RATE ASSESSMENT: MONITORING BY AUSCULTATION. J.C. Morrison, B.F. Chez,[X] I.D. Davis,[X] J.R. Allbert,[X] R.W. Martin, W.E. Roberts, J.N. Martin, Dept. Ob/Gyn, Univ. Mississippi Med. Ctr., Jackson, MS

Objective: To determine if intermittent auscultation of fetal heart rate (FHR) using strict frequency of evaluation and documentation criteria is feasible under clinical conditions in a busy labor and delivery suite in a tertiary center. Patient Population: During a 3-month period, 862 consecutive women in labor with live fetuses between 24 and 43 weeks' gestation were available for intrapartum auscultation of FHR (case series study design). Main Outcome Measured: Whether or not auscultation could be initiated and maintained for evaluation of FHR during the intrapartum period with a frequency requirement of auscultation and documentation of 15 minutes in the first stage of labor and 5 minutes during the second stage. Results: In 420 patients, this modality was not begun due to inability of the nurses to meet 1:1 staffing requirements. In 19 patients, auscultation was not performed due to obesity (12) or patient refusal (7) while in the remaining 423 patients it was begun. Auscultation was initiated during a contraction and extended for 30 seconds after the uterine activity ceased. It was repeated every 15 minutes in the first stage and 5 minutes in the second stage of labor. Of the 423 assessed by auscultation, 392 were unable to complete monitoring due to the frequency requirement (n=212) or the recording criteria (n=163). Of the 31 patients where ausculation was used successfully, labor was < 6 hours in 22 patients. In 9 patients who successfully completed ausculation, there was a 1:1 nurse ratio during the entire labor. Conclusion: Auscultation offered during the intrapartum period using stringent evaluation and recording criteria is not feasible under normal labor and delivery suite conditions unless a 1:1 nursing ratio is always available. This degree of staffing is rarely possible for the majority of obstetric areas in the United States during the entire labor.

138 EFFECTS OF MAGNESIUM SULFATE ON FETAL HEART RATE MONITORING IN THE PRETERM FETUS. JW Wright,[1,2] BD Wright,[1X] LE Ridgway,[2] DL Covington,[1X] JR Bobitt.[1] [1]Area Health Educ. Ctr, Wilmington, NC; [2]Univ. of Texas Health Science Ctr at San Antonio

Previous studies have reported that magnesium sulfate ($MgSO_4$) alters important characteristics of fetal heart rate monitoring. However, the results of these studies are inconsistent and most used preeclamptic patients near term. Because preterm labor is associated with other high risk conditions, the effects of $MgSO_4$ tocolysis on fetal heart rate monitoring are clinically important. We sought to evaluate these effects using 1) objective criteria with decreased variability defined as oscillation band width <6 bpm, normal variability as 6-25 bpm, and increased variability as >25 bpm; and 2) subjective evaluation with variability graded as absent, decreased, normal or increased. Agreement of 2 of 3 blinded examiners was used to define the categories. We prospectively collected fetal heart rate tracings for 30 minutes before and after $MgSO_4$ loading in 48 preterm labor patients. We compared pre- and post-therapy results using McNemar's test for categorical data and a paired t-test for baseline fetal heart rate. No monitor tracings were read as having absent or increased variability.

Results	Pre (n=48)	Post (n=48)	p
Baseline FHR	140.8	137.3	.001
NST Reactive	26	21	NS
Objective Variability			.13
Band width 6-25	48	44	
Band width <6	0	4	
Subjective Variability			.006
Normal	46	36	
Decreased	2	12	

Subjective evaluation demonstrated a greater likelihood of decreased variability after $MgSO_4$ loading. However, loss of variability was not shown by objective measurement. We conclude that $MgSO_4$ tocolysis may be associated with subjectively decreased variability.

139 FETAL PULSE OXIMETRY DURING LABOUR

J Gardosi[X], C Schram[X], D Damianou[X], EM Symonds[X]
Perinatal Research & Monitoring Unit,
Queens Medical Centre, Nottingham, England

Pulse oximetry, the non-invasive measurement of arterial oxygen saturation, has become a principle form of monitoring in several specialities. In perinatology, its application has been hampered by poor access to the fetus. We have developed a fetal oximeter probe which is held on the scalp by means of a double Copeland clip. The probe can be applied from 2 cm cervical dilatation and a continuous, on-line trend of oxygen saturation can be recorded straight onto the cardiotocograph paper. Preliminary results (105 labours) showed that saturation values on the fetal scalp present a stable baseline during normal labour (70 - 90%, median 82%). An unstable trend and a fall to below 60 % saturation was observed during the development of fetal acidosis. Our in vitro and animal studies have confirmed that fetal Hb dissociation curves are sensitive to the Bohr shift and give lower oxygen saturation levels during acidosis. Artifacts may arise due to non-arterial pulsations - e.g. from caput - and we have shown that this error can be excluded by signal processing which analyses both electronic (ECG) and optic (plethysmographic) signals and rejects non-synchronised readings. Incomplete apposition of the probe results in false-low readings; we have quantified this error and shown that the AC and DC components of the oximeter signal are affected differentially, thus affecting the calculations by the oximeter. Our probe failed in the presence of thick fetal hair, and a new probe is being developed which avoids this problem by apposing sensors to fetal skin away from hairy scalp. Pulse oximetry holds enormous promise for improving fetal surveillance during labour but successful adaptation requires attention to the particular difficulties and errors that may arise from this new application.

140 MECONIUM: MARKER FOR HIGH-RISK PREGNANCIES. M. Berkus, A. Samueloff,[X] E. Xenakis,[X] N. Field,[X] L. Ridgway, O. Langer. Dept. OB/GYN, The Univ. Texas Health Science Center, San Antonio, TX.

Traditionally, meconium (MEC) portends impending or ongoing fetal compromise, but recently this association has been questioned. Controversy exists regarding the relationship of MEC with abnormal fetal heart tracings, Apgar scores, pH and outcome. In this study, we sought to characterize the outcome of the patient with MEC-stained amniotic fluid. Over 2,000 consecutive deliveries were analyzed by a team of perinatologists, including scoring of the cardiotocograms (after Krebs). The incidence of term (>2500 g) babies with moderate or thick MEC was 16.7%. Mothers with chorioamnionitis and gestational diabetes had an increased incidence of MEC (35.6% and 21.4%, respectively; p<.03). In contrast, no significant increase in MEC was found in SGA (14.3%), hypertensive (14.4%) or prolonged pregnancies (19.4%) when compared to the non-MEC group. Infants with MEC were found at significantly increased risk for:

	MEC (%)	NOMEC (%)	RR (95% CI)
1st stage FHR abnormal	20.9	13.5	1.5 (1.2 2.0)
2nd state FHR abnormal	49.8	20.2	2.5 (1.9 3.1)
APGAR 1" <7	15.4	5.8	2.7 (1.7 4.1)
APGAR 5" <7	2.5	0.5	5.0 (1.6-15.6)
pH<7.2	26.9	13.6	2.0 (1.4 2.7)
Sepsis	4.1	1.1	3.6 (1.6-8.1)
Oxygen support	9.2	3.0	3.1 (1.2-8.0)
NICU admission	13.5	2.9	4.6 (1.9 11.3)

The study also revealed that MEC infants were more likely than non-MEC to: 1) be delivered by emergency C/S (15.4% vs 9.8%, p<.03); 2) experience adverse neonatal outcome (8.3% vs 3.9%, p<0.2), as defined by NICU stay, respiratory distress, or abnormal neurological sequelae; and 3) have significantly more adverse outcome when associated with bradycardia or decreased variability (p<.03), but not with tachycardia or lack of accelerations. Our data suggests that, even with modern OB care, a pregnancy with meconium is at increased jeopardy and should be considered high risk.

141 A NEW DIMENSION IN UMBILICAL CORD BLOOD pH: NEONATAL pH. K.G. Goldaber,* K.J. Leveno, L.C. Gilstrap III, M.A. Kelly,* M.L. Sherman,* Dept. Ob/Gyn, Univ. Texas Southwestern Med. Ctr., Dallas, Texas

Umbilical cord blood gases are increasingly being used in the evaluation of infant outcomes. Logically, infant outcomes should also be measured in relation to acid-base events occurring during the neonatal period, especially the first hours of extrauterine adaptation. We correlated umbilical artery blood pH to arterial pH measured within 2 hours of birth in 476 high-risk infants. Results are summarized in the following table showing the number of infants within cord blood pH groups and their corresponding initial neonatal pH:

	Neonatal pH					
Cord pH	<7.00	7.00-7.09	7.10-7.19	7.20-7.29	7.30-7.39	≥7.40
<7.00	3	6	3	4	1	0
7.00-7.09	1	2	2	8	6	1
7.10-7.19	1	0	7	21	32	4
7.20-7.29	0	3	20	87	72	21
7.30-7.39	0	7	9	50	67	16
≥7.40	1	1	1	10	5	4

The shaded numbers include 42 (9%) infants with cord blood pH values 7.20 or greater and neonatal values 7.19 or less. 12 (3%) of these infants had neonatal pH values 7.10 or less. Conclusion: Acidemia can occur in the immediate newborn period.

142 THE EFFECT OF MAGNESIUM SULFATE ON FETAL HEART RATE VARIABILITY IN THE TERM PREECLAMPTIC PATIENT. L.E. Ridgway, O. Langer, A. Samueloff,[X] M.D. Berkus, N.T. Field,[X] E. Xenakis,[X] Dept. of Ob/Gyn, UTHSC, San Antonio, TX.

Controversy exists regarding changes in fetal heart rate (FHR) variability associated with use of magnesium sulfate ($MgSO_4$). We investigated changes in FHR variability associated with $MgSO_4$ infusion. FHR tracings of 1500 consecutive deliveries were prospectively analyzed by blinded MFM specialists in 2 time windows: 1) first 30 min period after admission to L&D and 2) a 30 min window 1 h prior to 2nd stage. Variability was defined by: 1) amplitude–from the baseline and 2) oscillations–number of times the FHR crossed the baseline, both defined in the best 1 min period of the window. For analysis, patients were stratified into 2 groups: 1) preeclamptic patients (PRE) with gestational age (EGA) ≥36 wks who did not receive narcotics and had normal outcome (n=57), and 2) non-hypertensive subjects with EGA ≥36 wks who did not receive narcotics and had normal outcome (NL; n=852).

	Time 1		Time 2†	
	Amplitude bpm	Oscillations	Amplitude bpm	Oscillations
Group I PRE, n=57	7.3 ± 2.6*	6.5 ± 1.9	6.4 ± 3.2*	6.4 ± 2.7
Group II NL, n=852	7.8 ± 3.2	6.8 ± 2.1	7.6 ± 3.3	7.0 ± 2.6
p	ns	ns	<.01	ns

*p<.05; †Group 1 at Time 2 on $MgSO_4$ infusion

We further analyzed the relations of amplitude in PRE and NL: a) during Time 1 comparable rates of normal amplitude were found between PRE (100%) and NL (96%); b) in contrast, a 3 fold increase in the rate of reduced amplitude (<3 BPM) was found in the PRE (14%) when compared to NLs (5%, p=.01); c) comparing Times 1 and 2 in PREs, there was a change in reduced amplitude from 0% to 14% (p=.006); and d) no significant difference exists in the incidence of reduced variability comparing Times 1 and 2 in NLs (4% vs. 5%). A similar significant change was found in oscillation even though there was no difference in the means. Thus, a physiological and clinical decrease in variability is seen with MgSO4 infusion.

143 PROLONGED BRADYCARDIA IN THE FIRST STAGE OF LABOR: WHEN TO DO WHAT. M. McFarland,* E. Xenakis,* O. Langer, A. Samueloff,* N. Field,* M. Berkus. Dept. OB/GYN, University of Texas Health Science Center at San Antonio, Texas.

It is generally accepted that prolonged fetal bradycardia in the 1st stage of labor signifies fetal compromise and warrants immediate intervention, but little exists in the literature to support this conclusion. We investigated the effect of 1st stage prolonged bradycardia, defined as a drop in the fetal heart rate of ≥30 bpm to ≤90 bpm for ≥2.5 min, on fetal outcome. 1500 consecutive deliveries were evaluated; the overall incidence of prolonged bradycardia was 4%. Fetal heart tracings were analyzed for 30 min prior to and after the episode of bradycardia for multiple parameters, e.g., presence or absence of accelerations, decelerations, variability and duration of bradycardia. 100 women (2:1 ratio) matched by gestational age and fetal heart rate score (after Krebs) served as controls. For purpose of analysis, newborns were stratified into outcome groups: good (infant went home in 2.5 days, was not admitted to the NICU); and adverse (NICU admission with respiratory support and/or other complications, i.e. hypotonia, IVH, sepsis or neonatal death). Overall, a significantly greater number of infants had low arterial cord pH (<7.2) in the bradycardia group (47.2% vs. 25.5%, p<0.05). Further analysis, evaluating outcome by mode of delivery, is shown below:

	Bradycardia		Control	
	C/S	Vag	C/S	Vag
Good	81.1%	88.9%	77.4%	97.1%
Adverse	11.5%	18.5%*	22.6%	2.9%*

*p<0.05

Finally, stepwise logistic regression was used to evaluate fetal heart rate components in order to predict adverse outcome. The total number of accelerations and late decelerations explain 38% of the variance. Duration of the bradycardia, time to recovery, baseline and variability were not found to be significant predictors. Our data suggest that although presence of prolonged bradycardia is not an indication for immediate delivery, caution should be exercised when allowing these patients to continue labor.

144 THE INCIDENCE OF INTRAPARTUM FETAL DISTRESS IN COCAINE ABUSING GRAVIDAS. K. Piacquadio[X], O.W. Jones III[X], P. Herbig[X], R. Resnik, Dept. Repro. Med. UCSD, San Diego, CA.

Women between the ages of 18-34 comprise 15% of the regular cocaine users in the U.S. Previous studies have shown that cocaine use during pregnancy is associated with abnormal fetal growth, preterm labor and delivery and an increased incidence of abruptio placenta. The purpose of this study was to determine if maternal cocaine use was correlated with an increased incidence of intrapartum fetal distress. The fetal heart rate and uterine contraction tracings from 75 women in labor with positive urine toxicology for cocaine on admission were read by two blinded observers. These tracings were compared with those of 166 women with no antepartum cocaine use. Data were analyzed by Group t test, Chi square or Fischer's Exact test where appropriate. The results of this study showed a significantly lower gestational age (p<0.01), birth weight (p<0.01) and one minute Apgar score (p<0.01) among cocaine-using gravidas as well as increased gravidity (p<0.01) and parity (p<0.01) when compared to the control group. The fetuses of the cocaine users had a significantly higher incidence of non-reactive tracings (p<0.01) and one observer found significantly decreased beat-to-beat variability (p<0.05). Neither observer found a significant change in baseline fetal heart rate or incidence or severity of variable or late decelerations. Arterial cord pH values were obtained in 26 of the cocaine exposed and 19 of the control fetuses. There was no significant difference between the two groups (cocaine pH 7.26 ± 0.11, control pH 7.24 ± 0.09). The results of this study suggest that cocaine use is not associated with significant intrapartum fetal distress.

Poster Session II

Thursday, February 6, 1992
4:00 p.m.-6:00 p.m.

Grand Salons I-IV

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Diagnostic Ultrasound	145-197
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145 **IS THE 5-STEP ECHOCARDIOGRAPHIC APPROACH FEASIBLE THROUGHOUT PREGNANCY?**

Danielle M. Blake, E. Albert Reece, Dimitrios Mastrogiannis[X]. Department of Obstetrics and Gynecology, Temple University School of Medicine, Philadelphia, PA.

Fetal echocardiography utilizes various ultrasound modalities for detailed heart study. The five principle views which are considered essential for a 2-D realtime cardiac study include the 4-chamber view, ventricular outflow tracts, cardiac inflow vessels, aortic and ductal arches, and the long and short axis great vessels. However, a complete echocardiogram is thought to be hampered by advanced gestational age. This claim, however, has not been subjected to study. An ongoing prospective study was therefore initiated to address this issue. All patients undergoing an ultrasound examination were invited to participate in this study. The five principle cardiac views were attempted by two operators. Three groups of gestational ages were analyzed: Group 1, 20-24 weeks; Group 2, 25-32 weeks; Group 3, 33-40 weeks. To assess the degree of difficulty in obtaining each view in each of 3 gestational age groups the length of time in minutes was determined. Our preliminary data summarized in the table, demonstrate the feasibility of obtaining these basic views throughout pregnancy and showed a lack of significant difference in time spent per study in either the second or third trimester.

	Time (minutes) Gestational age groups		
Cardiac views	1	2	3
4 chamber view	1.33± .96	1.65±2.11	.81± .62
ventricular outflow tracts	1.50± .42	1.38± .41	1.30± .39
cardiac inflow vessels	.67± .30	1.65±1.34	3.48±2.95
aortic & ductal arches	2.87±2.88	3.82±2.30	2.33± .88
long/short axis GV	2.37± .78	3.03±1.75	3.47±2.63
all views	8.73±1.02	11.53±5.91	11.40±3.40

CONCLUSION: Although additional time is always spent and other ultrasound modalities are used reviewing cardiac structure and function in detail, the above data indicate that the 5 step approach to 2-D realtime fetal echocardiac exams is applicable throughout pregnancy. Therefore, advanced gestational age should not be considered a precluding factor to a complete cardiac exam.

146 SONOGRAPHIC DETECTION OF DECREASED FETAL HEAD GROWTH SECONDARY TO ALCOHOL AND COCAINE EXPOSURE. S.S. Martier,[X] R.J. Sokol, J.W. Ager,[X] Dept. Ob/Gyn, Wayne State Univ /Hutzel Hosp., Detroit, MI

Decreased head circumference (HC) at birth has been previously reported to be associated with prenatal alcohol and drug exposure. In a large study on prenatal alcohol exposure, approximately 10,000 Black gravidas were interviewed prospectively in a core city prenatal clinic. To examine the relationship, if any, of decreased HC *in utero* to polydrug exposure, a sample of 1,137 consecutive gravidas who had ultrasound examinations at 28.5 ± 7.6 weeks gestation as part of their antenatal care was evaluated. Head circumference *in utero* (HCIU) was calculated using morphometric measurements of head biparietal diameter (BPD) and occipito-frontal diameter (OFD) measures taken *in utero* (3.14 × (1.5 + BPD + OFD)/2). HCIU was residualized for gestational age at time of ultrasound exam on the part of the sample unexposed to drugs or heavy alcohol, i.e., ≤.5 ounces of absolute alcohol per day, and then applied to the entire sample. In multiple regression analyses, exposures (alcohol, cannabis, cocaine, narcotics) and covariates (maternal age, prepregnancy weight and cigarette smoking) were related to HCIU. The proportion of drinking days reported at the time of conception and use of cocaine related significantly to smaller HCIJ ($F(2,1136) = 9.9$; $R^2 = 1.7\%$). This finding supports the hypothesis of an adverse impact of prenatal cocaine and alcohol exposure on decreased head growth which has been associated with increased risk of aberrant neurobehavioral development. These findings suggest that effects of heavy maternal alcohol and cocaine use may be detectible *in utero*, closer to the time of the insult.

147 FETAL FEMUR LENGTH: THE IDEAL ASSESSMENT OF GESTATIONAL AGE? A RETROSPECTIVE AND PROSPECTIVE ANALYSIS OF TWO DISTINCT RACIALLY DIFFERENT POPULATIONS. H.O. Thompson, M.D.,[X] J.S. Abramowicz,M.D., C.Cox,Ph.D,[X] Depts. Obs/ Gyn and Biostatistics, University of Rochester Medical Center, Rochester, New York.

Exact determination of gestational age (GA) and assessment of fetal growth are two primary tasks of prenatal care providers. Femur length(FL) is less affected than other feta. measurements in growth disturbances. The data is inconclusive on the effect of race on biparietal diameter(BPD), abdominal circumference(AC), FL and birth weight(BW) with respect to GA primarily due to sample size and poorly defined populations. To evaluate the hypothesis that there are racial differences,AC, BPD, FL and BW were compared in a cross-sectional study (24-43 weeks) in 1401 singleton, live, black(507) or white(894) infants born within seven days of an ultrasound examination. The statistical analysis consisted of X^2, unpaired t-test, ANOVA, and analysis of covariance (ACOVA) with regression coefficients comparisons(RCC) with GA as the independent variable and parity, race and fetal sex as modifiers. The maternal and fetal demographic variables were similar in the two groups. The mean BW and BPD in black fetuses were smaller($p=0.05$ and 0.009) while there were no difference in the FL and AC. Males had significantly heavier BW and larger mean BPD than the females ($p=0.019$ and 0.010) while there were no differences in FL or AC. White males weighed more with the largest mean BPD while black females were lightest with the smallest mean BPD (ANOVA $p=0.037$, $p=0.004$). The ACOVA and the RCC confirmed that there was an effect of race on BPD, AC and BW with respect to GA while FL was uneffected ($P=0.009, <0.001, <0.001$). A prospective analysis was performed on a second sample of 195 fetuses(88 Black and 107 White) using the models developed during the ACOVA of the first population with Similar results. This suggests separate GA equations may have to be developed for each race if parameters other than FL are used. FL may be the measurement of choice to determine gestational age regardless of maternal race.

148 **TRANSCEREBELLAR MEASUREMENTS IN TWIN PREGNANCIES AND THE EFFECT OF INTRAUTERINE GROWTH RETARDATION.** L. Lettieri, A. Vintzileos, D. McLean, J. Rodis, W. Campbell, E. Wolf, Univ. of CT Health Ctr, Farmington, CT

Transcerebellar diameter (TCD) and transcerebellar diameter/abdominal circumference (TCD/AC) measurements have been established for singleton, but not for twin pregnancies. In singleton pregnancies, controversy exists on whether or not cerebellar growth is affected by intrauterine growth retardation (IUGR). We undertook this retrospective study of 171 patients with twin pregnancies in order to establish nomograms for both TCD and TCD/AC ratios in twin fetuses and to analyze the effect of growth retardation on these measurements. The data was analyzed in a cross-sectional manner. The TCD measurements for twin A & B were grouped together after analysis revealed no difference in TCD between the twin pairs (mean TCD (±SD): twin A 2.57 (±.86) vs. twin B 2.56 (±.85), $p=.8$). Of 342 fetuses, 322 were available for analysis. The gestational age ranged from 14 38 weeks. The nomograms were established from these fetuses. The TCD increased linearly with gestational age ($r=.94$, $p<.001$). The TCD/AC ratio was stable throughout gestation ($r=.005$), with a mean TCD/AC (±SD) of 13.7 (±1.2). The 5th and 95th percentiles were 12.0 and 15.9, respectively. Twenty-four twin fetuses with IUGR (EFW ≤ 5th%ile by Yarkoni) were then analyzed; 10 (42%) of these fetuses had a TCD of ≤5th%ile for gestational age. The mean TCD/AC (±SD) of the IUGR fetuses was 14.6 (±1.6). However, only 4 of the 24 IUGR fetuses had an abnormal TCD/AC ratio for a sensitivity of 17%. The specificity, positive and negative predictive values were 95%, 21% and 94%, respectively. All fetuses with a TCD ≤ 5th %ile had a normal TCD/AC ratio. We conclude therefore, that an abnormal TCD/AC ratio is not very helpful (sensitivity of only 17%) in identifying growth retardation in twin fetuses. These findings also suggest that intrauterine growth retardation may affect cerebellar growth in twins since 42% of the IUGR fetuses had TCD of ≤5%ile.

149 PROSPECTIVE ANALYSIS OF MALFORMATIONS DIAGNOSED BY ULTRASONOGRAPHY IN CHROMOSOMALLY ABNORMAL FETUSES. B.R. Elejalde, [X]J.M. Acuña, [X]M.M. de Elejalde, Medical Genetics Institute, S.C. Milwaukee, WI.

2,547 patients underwent prenatal diagnosis, 15 had a chromosomal abnormality other than trisomy 21. 6 had trisomy 18, 4 were 45,X, 2 were triploidies, 1 had trisomy 9, 1 was a trisomy 13 and 1 had a ring 14. 147 abnormalities were found by ultrasonography (US) before the karyotype was known. 203 abnormalities were found postnatally. The 56 that were not found by US were divided into: 1) recognizable by US (9), 2) recognizable under special circumstances (14), 3) not recognizable by US (33) and 4) not recognizable postnatally (9). Out of 156 diagnosable by ultrasound, 147 (94.2%) were diagnosed. If those recognizable under specific circumstances are included, 147 out of 170 (86.5%) were diagnosed. Syndromic diagnosis, before karyotyping, was correctly done in 4 trisomies 18, 2 triploidies, 1 trisomy 13 and 3-45,X. The above distribution of abnormalities defines ultrasound resolution and the bases for quality control

150 PROSPECTIVE STUDY OF THE ULTRASONOGRAPHIC RESOLUTION OF NORMAL FETAL CHARACTERISTICS. B.R. Elejalde, [X]M.M. de Elejalde. Medical Genetics Institute, S.C. Milwaukee, WI.

3,900 patients underwent 8,200 ultrasonographic examinations. Fifty one structures were measured, 68 ossification centers and 173 anatomical structures were scored as: seen, not clearly seen, not seen, not present and abnormal. Nineteen patterns of fetal activity were similarly recorded and their frequency scored. Only normal pregnancies 10 to 39 weeks were included. Descriptive statistical analysis was used for the analysis of each one of the 311 items per week. Centiles 1st to the 99th were established for each measurement and week. A table including the characteristics for each week based on this analysis was constructed and constitutes the bases to determine the mean and the range of expected results of the fetal ultrasonographic examinations at weeks 10 to 39. They define the bases to determine the levels of ultrasound resolution. Resolution is affected by the knowledge of the operator, obesity and fetal position.

151 AMNIOTIC FLUID VOLUME ASSESSMENT IN HUMAN PREGNANCY: COMPARISON OF SONOGRAPHIC ESTIMATES VERSUS DIRECT MEASUREMENTS USING A DYE-DILUTION TECHNIQUE. Gary Dildy, Noe Lira[X], Kenneth Moise Jr, Gerry Riddle[X], Russell Deter[X]. Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas.

Abnormalities in amniotic fluid volume (AFV) are associated with poor perinatal outcome. The purpose of this study was to compare the accuracy of indirect real-time sonographic techniques of AFV assessment with a direct technique of AFV measurement. METHODS: A water-soluble dye (aminohippurate sodium) was instilled into the amniotic sac of women undergoing amniocentesis for fetal lung maturation during the third trimester. Thirteen distinct semiquantitative sonographic techniques in both the supine and lateral recumbent positions, including the amniotic fluid index (AFI), were performed. A second amniocentesis was performed to determine dye concentration in order to calculate actual AFV. Optimal polynomial regression functions of sonographic measurements (independent variable) versus actual AFV (dependent variable) were determined. Predicted AFV was calculated by introducing the value of the sonographic measurement into the equation. The percent difference between the actual and predicted AFV was determined for each measurement, and the mean, standard deviation, and 95% range were calculated. The Student t-test, ANOVA, and F-test were used to determine differences between techniques, with $p<0.05$ considered significant. RESULTS: 50 patients were enrolled into the study. Mean actual AFV as determined by the dye technique was 1066 ml (range 129 to 4444 ml). Mean AFI was 16.4 cm (range 2.7 to 31.3 cm). The regression equation for the AFI technique was: $y = -713 + 258x - 17.1x^2 + 0.437x^3$; $R^2 = 72.1\%$, mean percent error = 11.5%, standard deviation of percent error = 53.0%, and 95% range of percent error was -51.7 to 103.7%. Among the 13 techniques, there were no differences in mean error as determined by ANOVA. Four of the techniques showed a statistically significant reduction in standard deviation of error as determined by the F-test, when compared to the AFI. CONCLUSIONS: The AFI overestimated the actual AFV by as much as 104 % at low ranges and underestimated the actual AFV by as much as 52 %, especially at higher ranges. Although there were statistically significant reductions in measurement error among several of the sonographic methods compared to the AFI, these differences did not appear sufficient to recommend changes in current clinical practice.

152 SERIAL HOURLY FETAL URINE PRODUCTION IN FETUSES WITH BILATERAL HYDRONEPHROSIS. SJ Carlan, D Adkins[1], M Gore[1], D Mastrogiannis, Depts Ob/Gyn U of S FL,Tampa, FL, ORMC, Orlando.

Since some ultrasound detected fetal hydronephrosis,(H), cases are not present after birth, it is possible that this finding may be functional and related to the greater urine production in the fetus compared to the neonate. The purpose of this study was to compare hourly fetal urine production (HFUP) in fetuses with bilateral H to a group of normal fetuses. Fourteen normal fetuses and five fetuses with bi-lateral H were scanned serially from 20 wks until delivery, and HFUP measurements were attempted. All fetuses with H had major calyceal system dilatation with kidney longitudinal length\ collecting system length ratios >30% for both kidneys throughout gestation. There were no detectible cases of hydroureter or extrarenal anomalies in either group. There was no difference in fetal kidney length or AFI during gestation. Glucose screens were performed on all women, and there were no maternal disorders in either group. There was no significant difference in maternal age,GTPAL,smoking or C-section rate. Mean BW was 2979 and 3569 for the H group and normal group respectively. Neonatal urologic workup in the H group was normal in all cases except one infant with persistent hydronephrosis (this fetus also demonstrated minor calyceal dilatation).

SERIAL HFUP cc/hr (MEAN ±1SD)

WKS	N	Bilateral H	N	Normal	P
20	5	3.6 ± 1.1	14	4.1 ± 1.9	NS
22	5	4.8 ± 1.2	14	5.0 ± 1.7	NS
24	5	9.8 ± 2.7	14	8.3 ± 2.7	NS
26	5	12.2 ± 4.1	14	10.6 ± 3.6	NS
28	5	12.4 ± 3.0	14	15.5 ± 4.2	NS
30	4	20.5 ± 4.6	13	23.6 ± 4.7	NS
32	4	24.7 ± 4.1	13	28.9 ± 5.6	NS
34	4	24.6 ± 3.8	13	37.3 ± 12.2	NS
36	3	37.1 ± 7.7	13	43.8 ± 13.9	NS
38	3	39.2 ± 8.3	8	58.3 ± 21.9	NS
40		]	8	47.9 ± 12.9	

We conclude that fetuses with ultrasound detected bilateral H have similar HFUP as fetuses without bilateral hydronephrosis.

153 ESTIMATED FETAL WEIGHT IS A POOR PREDICTOR OF MACROSOMIA AND SHOULDER DYSTOCIA.

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We evaluated the utility of ultrasound estimated fetal weight (EFW) to predict macrosomia (birthweight >4.0 kg) and shoulder dystocia (SD) in 566 patients (53 diabetics) with EFW performed within 28 days of delivery. 7 SD's occurred in the study group. EFW was determined by three methods (Shepherd, Hadlock, Spinnato) and projected to the date of delivery using a standard fetal growth curve. Mean error of EFW was 17.9 g overestimation (1 standard deviation = 0 348 g); mean absolute error was 8.8% of birth weight. Receiver operator characteristics of EFW to predict macrosomia were plotted. EFW > 4.0 kg was less efficient for predicting macrosomia in diabetic patients; non-diabetics: sensitivity = 0.60, specificity = 0.96; diabetics: sensitivity = 0.33, specificity = 0.96.

Birth-weight	Total N	Vaginal Deliveries N	EFW>4kg	SD	SD+EFW>4kg
<3 kg	308	222	0	0	0
3 - 4 kg	289	199	11	4	1
>4 kg	69	32	15	3	2

A policy of primary cesarean delivery for EFW >4.0 kg would have resulted in 26 additional cesarean sections and prevented only 3 SD's (40%). We conclude that EFW is a poor predictor of macrosomia and SD. Recommendations to perform primary cesarean section for macrosomia based on studies utilizing birthweight, a parameter unknown to obstetricians prior to delivery, are of little clinical value.

154 ANTEPARTUM ULTRASOUND ASSESSMENT IN TWIN GESTATIONS AS PREDICTOR OF FETAL COMPROMISE

B. Campbell, R. Newman, L. Laham[X], J. Ellings[X], D. Eller, Med Univ of S.C., Charleston, S.C.

Multifetal gestations are associated with rates of perinatal mortality that are 5 to 10 times higher than seen with singleton gestations. Because of the risk inherent to multifetal gestations, appropriate antepartum surveillance is intensely debated but poorly studied. Besides prematurity, the majority of complications which contribute to this excessive perinatal mortality can be diagnosed by ultrasonography (US) including twin discordancy, IUGR, oligohydramnios, polyhydramnios, monoamniotic twins, and anomalies. The current study was designed to assess the ability of monthly antepartum ultrasound examinations to predict multifetal gestations at risk for intrauterine compromise. One hundred six twin gestations were followed in a special antepartum twins clinic from 1-1-88 to 7-31-91. The mean gestational age at diagnosis of these twins and their mean gestational age at delivery were 18.7 ± 6.6 weeks and at 35.6 ± 2.9 weeks respectively. Ultrasound examinations were performed every 4 weeks. Of the gestations followed, 91 (86%) had an US performed within 4 weeks of delivery. Non-stress testing was begun at 34 weeks unless otherwise indicated. The US was considered normal if there was no evidence of IUGR (≤10th percentile), fetal discordance (≥25), or fetal anomalies and an intervening membrane and were normal amniotic fluid were noted. Seventy-seven percent had a normal US and 23% had at least one abnormal finding. Pregnancy outcome was then compared for the women with normal vs. abnormal antepartum ultrasound examinations. The incidence of antepartum, intrapartum, or neonatal compromise defined by fetal distress, five minute APGAR <7, cord arterial pH less than ≤7.10, meconium aspiration or neonatal seizures was 11 of 23 (48%) in the abnormal US group which was significantly higher than the 4 of 78 (5%) among the normal US group. (P < 0.001). Of those with the abnormal US findings, 36% had abnormal antepartum fetal heart rate testing compared to only 1.3% of the normal US group. The incidence of preterm labor or preterm rupture of membranes was not different between the two groups. The study suggests that serial ultrasonography effectively identifies abnormal multifetal growth and development and is an important screening tool to identify multifetal gestations at risk for utero placental insufficiency and in need of more intensive antepartum surveillance.

155 ROUTINE AMNIOCENTESIS IS NOT INDICATED TO EVALUATE AN UNEXPLAINED ELEVATED MATERNAL SERUM ALPHA-FETOPROTEIN.

M Albini[X], W. Cusick[X], J. Rodis, L. Lettieri, M. McMahon[X], A. Vintzileos, University of Connecticut Health Ctr, Farmington, CT.

Although routine amniocentesis is the standard of care as part of the evaluation for an elevated second trimester maternal serum alpha-fetoprotein (MSAFP), we do not routinely perform this procedure when investigating an elevated MSAFP. Our management plan consists of a targeted ultrasound examination with amniocentesis offered selectively as indicated by our ultrasound. A retrospective analysis was conducted of all targeted ultrasound examinations performed for evaluation of an elevated MSAFP at the Connecticut MSAFP Screening Program from November 1988 to June 1991. An elevated MSAFP was considered to be greater than or equal to 2.0 multiples of the median (MOM). A total of 392 patients were included in the study population. The mean gestational age at presentation was 20.3 weeks and the average MSAFP was 2.73 MOM. The final outcome was obtained of 379 patients (96.6%) and this group constitutes the focus of our report. Nine fetal-placental anomalies (2.4%) were detected by ultrasound which accounted for the elevated MSAFP: omphalocele (3); anencephaly (2); gastroschisis (1); myelomeningocele (1), encephalocele (1) and placental abruption (1). All nine patients underwent an amniocentesis for fetal karyotyping. One fetus with an omphalocele was proven to have trisomy 18. The remaining 370 patients (97.6%) were left with an unexplained MSAFP elevation. Forty-four patients (44/370 or 11.8%) underwent an amniocentesis due to inadequate fetal visualization or maternal preference. There was one pregnancy loss (1/44 or 2.3%) within 4 weeks of the amniocentesis. No abnormal fetal karyotypes were identified in these 44 patients. Also, no phenotypically abnormal infants were observed in the 326 patients who did not undergo an amniocentesis. Most importantly, no fetal anomalies were missed by ultrasound (sensitivity 100%). We conclude that amniocentesis is not routinely indicated for the evaluation of an unexplained MSAFP elevation.

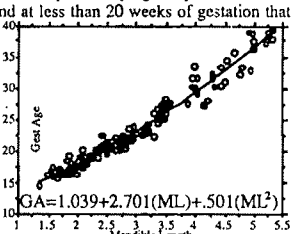
156 ENDOLUMINAL CATHETER-ASSISTED TRANSCERVICAL ULTRASOUND OF FIRST TRIMESTER PREGNANCY.

N. Ragavendra,* M. B. Beall, J. McMahon,* E. G. Grant.* Depts. of Radiology and Ob/Gyn, UCLA Medical Center, Los Angeles, Ca.

Transvaginal sonography is an excellent technique for confirming the intrauterine location of pregnancy, and determining the presence of cardiac activity and age of the first trimester conceptus. However, its ability to display anatomic structures in the developing embryo is less than satisfactory. In order to enhance the visualization of anatomic structures of the early human embryo, we have utilized a commercially available, catheter-based, miniaturized ultrasound transducer (12.5 MHz) and introduced it into the gravid uterus through the cervix in twenty-five women about to undergo therapeutic abortions of first trimester pregnancies. In embryos of 6.5 - 8.5 menstrual weeks, transcervical ultrasound showed brain vesicles, limb buds, cardiac chambers, spine and in cases where umbilical cord was seen, blood flow was noted. Early detection of gross embryonic malformations is potentially possible with this technique.

157 BIOMETRY OF THE FETAL MANDIBLE. C. Otto, L.D. Platt, Dept. Ob/Gyn, Cedars-Sinai Med. Cntr./UCLA, Los Angeles, CA

The fetal mandible has not previously been studied in an objective fashion. A plane was established that measures one ramus of the jaw. The proximal landmark, the temporomandibular joint, is visualized below the level of the orbits in an axial section and the anechoic cartilaginous area between the mandibular rami, the symphysis mentis, is the distal landmark. One hundred and thirty two subjects were identified and studied in a cross sectional manner. All measurements were made by one author (C.O.) with commonly available ultrasound equipment. Patients studied were without known medical or obstetric complications and had a known last menstrual period(LMP) and either a positive pregnancy test within 6 weeks of the LMP or an ultrasound at less than 20 weeks of gestation that confirmed the LMP. No fetus studied had an abnormal karyotype or structural malformation identified at birth. Measurement of three separate images of mandible length were obtained and averaged. Statistical analysis was performed on a Macintosh II using Stat View II statistical software package. With the mandible length as independent variable, an equation for predicting gestational age was derived by polynomial least squares regression. There was a high degree of correlation with an adjusted R^2 of 0.963. The accompanying figure is a scatterplot of the data and the regression formula. An additional 31 fetuses were measured by the authors to evaluate interobserver variation. There was no significant difference in the measurements of the two observers.



Conclusion: The size of the fetal mandible correlates well with and may be used to predict gestational age. The landmarks of the measurement are reproducible and interobserver variation is acceptable. Further studies may allow delineation of growth of the fetal jaw in pathologic conditions and precise, objective determination of micrognathia.

158 BIPARIETAL DIAMETER PREDICTS NEONATAL SURVIVAL AS WELL AS ACTUAL BIRTH WEIGHT IN THE 500-1,000 GRAM INFANT. RS Smith[x], and SF Bottoms. Wayne State Univ., Hutzel Hospital, Detroit, MI.

Determining neonatal prognosis for the severely premature fetus is difficult but important in counseling and guiding obstetrical management. The obstetrician must rely on survival rates based on birth weight; error in estimated fetal weight limits the confidence regarding prognosis before delivery. In an attempt to improve the accuracy of prenatal predictions of survival we studied 130 singleton liveborn infants having birth weights of 500-1,000 grams and complete ultrasound examinations within 3 days of delivery. Birth weights were uniformly distributed. 80 infants survived; 50 died. For purposes of comparison, estimated fetal weight, biparietal diameter (BPD), femur, birth weight, and pediatric estimate of gestational age were evaluated as screening tests for neonatal survival using receiver-operator characteristic (ROC) curves. In this model, sensitivity and false positive rate were calculated using each value of every parameter as a cut point to predict survival. Visual inspection of the ROC curves indicated that BPD was the best single predictor. Prognostic tables based directly on obstetrical parameters were generated. Among infants < 800 grams, discriminant function analysis revealed that cesarean birth was associated with a higher survival rate, and that underestimation of fetal weight was linked with a lower cesarean rate and a worse prognosis. Irrespective of controlling for route of delivery, BPD did as well as actual birth weight and all other parameters in predicting survival. We speculate this is due to BPD being less subject to variation in growth; it may provide the most accurate estimate of gestational age in this weight range.

159 ASSESSMENT OF EARLY FETAL GROWTH IN DIABETIC PREGNANCIES BY VAGINAL SONOGRAPHY. Laxmi Baxi, Tessie Tharakan[x], Ana Monteagudo[x], Ilan Timor. Dept. of Ob/Gyn, College of P & S, Columbia Univ. & Columbia Presbyterian Medical Center, New York,

Inadequate glycemic control early in diabetic pregnancies is associated with an increased incidence of congenital malformations. Pedersen has reported early growth delay in these patients. We prospectively studied early fetal growth in 37 diabetic and 30 control pregnancies, all of whom had at least two vaginal sonograms between 40 and 112 days of gestation. Glycosylated hemoglobin (HbA1) measurements reflected glycemic control in early pregnancy. A discrepancy of >0.5 wks. was labeled as early growth delay.

Control of Diabetes	Early growth delay		No growth delay		Tot
	No malform	*malform	*No malform	*malform	
Poor HbA1 >8.5	9	2	14	2	27
Good HbA1 <8.5	1	0	9	0	10
Total	10	2	23	2	37

*caudal regression syndrome, *bilateral hydrothorax, *tetralogy of Fallot, *megaloureter, *4 spontaneous abortions, X^2-3.147, P-ns.

Of 37 diabetic patients, 27 had elevated HbA1 (>8.5%), indicating poor control, in the first trimester. Eleven of these had growth delay (40.7%). Delayed growth was seen in 1/10 in patients with well controlled diabetes (P-ns). In conclusion, using vaginal ultrasound, we did not detect increased incidence of early growth delay in all diabetic pregnancies. However, the delay was significant in patients with suboptimal glycemic control as compared to non-diabetic patients (X^2-4.079, P<.05). *(Supported by a grant from the Diabetic Foundation)*

160 ABNORMAL AMNIOTIC FLUID VOLUME AND FETAL STRUCTURAL DEFECTS. H.M. Wolfe, M.P. Dombrowski, S.F. Bottoms, I.E. Zador*, R.J. Sokol, Dept. of Ob/Gyn, Hutzel Hosp./Wayne State Univ., Detroit, MI

To date, studies of amniotic fluid volume (AFV) have focused primarily on extremes of AFV (oligohydramnios (oligo) and polyhydramnios (poly)) and their association with specific anomalies. We studied 3456 patients at greater than 15 weeks gestation. Abnormal (abn) AFV, by subjective and objective criteria, was grouped as oligo, decreased (decr), increased (incr) and poly. Each was compared to normal AFV for sonographically detected abnormalities of major organ systems. Odds ratios and 95% confidence limits (CL) were calculated. As expected, oligo was associated with an increased frequency of GU anomalies (39.2x) and poly with GI (21.0x) anomalies. Interestingly decr AFV was associated with an increase in heart (40x) and GI anomalies (12.9x). Heart (19.4x), umbilical cord (3.8x) and GI (27.8x) anomalies were also more common with incr AFV as indicated below (*p < .05).

	Oligo	Decr	Nl	Incr	Poly
Stomach	0/48	0/236	1/3006	1/111*	0/19
Bladder	2/51*	0/234	0/2994	0/110	0/19
Kidney	2/49*	1/219	4/2848	1/111	0/18
Umbilical Cord	0/13	0/155	5/2396	1/107	1/18*
Heart	1/20*	6/125*	2/1589	2/84*	0/16
Intestines	0/40	3/209*	2/2944	2/109*	1/17*
Ventricles	1/51*	0/215	3/2288	0/107	1/17*
Total Odds Ratio:	18.9	6.3	1.0	11.0	26.6
95% CL	7.3-49.2	2.9-13.6		4.8-25.5	7.2-98.3

Overall, the risk of anomalies is 6-26x greater with any departure from sonographically normal AFV. In fact, the incidence among cases of oligo and poly is not significantly increased when compared to incr and decr AFV. AFV reflects a continuum of risk. Patients with abn AFV, even those not meeting formal criteria for poly or oligo, should be considered at substantially increased risk for anomalies and carefully evaluated prenatally for their presence.

161 **CLINICAL EVALUATION OF A FETAL GROWTH PREDICTION MODEL** J. Owen, S.P. Cliver, M. Hardin, F.L. Goldenberg, H.J. Hoffman. The University of Alabama at Birmingham

Because normative values for sonographically determined biometric parameters are generally population-based, the growth-disturbed fetus could be overlooked unless its measurements fall outside of a predetermined, usually arbitrary, range. The Rossavik growth model [$P=c(t)^{k+s(t)}$] has been reported to define the growth potential of individual fetuses based on their own biometric parameters obtained prior to 26 weeks' gestation. We sought to confirm the validity of the model in a population of 581 gravidas who were followed with serial sonography as part of an NIH-funded protocol of fetal growth. Overall the model predicted the fetal abdominal circumference (AC), femur length (FL), and head circumference (HC) on the last sonogram (mean GA=35.9 wks) prior to delivery (mean GA=38.6 wks) with very small mean errors (AC=-1.25%, FL=-0.91%, HC=-3.99%). We then examined the model in the context of growth-disturbed newborns. Observed deviations from the model's mean predicted growth values were computed for each biometric parameter and expressed as percentiles above and below the mean. Only 2 of 46 growth restricted newborns (<10th birthweight percentile) had a measured AC growth deviation (on their last sonogram) above the 90th percentile of the model's predictions (suggesting that the fetus was exceeding its potential) compared to 23 of 46 whose measurements were below the 10th percentile. Conversely, 20 of 45 large-for-gestational age infants (birth weights >90th percentile) had AC deviations above the 90th percentile, while none fell below the 10th percentile. We conclude from these observations that this growth model may be of value in the prenatal identification of the growth-disturbed fetus. We will report the utility of the model in this population with respect to other clinical parameters including ponderal index, skin fold thickness and perinatal complications.

163 THE INCIDENCE OF DUCTAL CONSTRICTION AND OLIGOHYDRAMNIOS DURING TOCOLYTIC THERAPY WITH IBUPROFEN. M.D. HENNESSY, M.D.*, E.C. LIVINGSTON, M.D.*, J. PAPAGIANOS, M.D.*, A.P. KILLAM, M.D., Duke Univ., Durham, NC

Indomethacin is being used increasingly in the treatment of preterm labor, however, it has been associated with up to a 50% incidence of fetal complications. Foremost among these is premature closure of the ductus arteriosus (DA) and oligohydramnios. Consequently, at our institution we began using Ibuprofen (1,200-2,400 mg/day) as a tocolytic agent up to 32 weeks gestation. Doppler echocardiography was performed within one week of instituting therapy and every 1-2 weeks thereafter until discontinuation of the Ibuprofen. Patients received biophysical profiles with evaluation of amniotic fluid every 1-2 weeks. Constriction of the DA was defined as a systolic velocity greater than 120 cm/sec with a diastolic velocity greater than 30 cm/sec. We retrospectively identified 52 pregnancies including 61 fetuses that were treated in this manner. There were no cases of maternal intolerance or morbidity secondary to the medication. There were no cases of oligohydramnios. There were 3 cases of low-normal fluid (Phelan index 5.0-8.0) that occurred after institution of Ibuprofen therapy and resolved after discontinuation. In 54 of 61 fetuses, adequate studies were obtained revealing only 4 (7%) with increased ductal velocities consistent with mild constriction. Three of these fetuses demonstrated constriction within one week of starting Ibuprofen. All had normal echocardiograms within one week of discontinuing therapy. There was no relation between the dosage of Ibuprofen used and the incidence of ductal constriction.

We conclude that Ibuprofen use before 32 weeks gestation in preterm labor has a low incidence of ductal constriction. Our study population did not demonstrate true oligohydramnics.

162 **DO SEMIQUANTITATIVE AMNIOTIC FLUID(AF) INDICES REFLECT ACTUAL VOLUME?** C.Croom,* B.Banias,* E. Ramos,* L.Devoe, A.Bezhadian* A. Hiett* Dept. OBGYN, Med. College of Georgia,Augusta,GA.

To determine how well ultrasound measures of maximal AF pocket depth(MAPD) and AF index (AFI) reflect actual AF volumes (AFVs), we studied 40 near-term patients, prior to amniocentesis for fetal lung maturity, who had **normal** AFV estimates by **"eyeball"** exam. Each patient had MAPD and AFI performed by the same examiner and received intraamniotic injection of a 10% paraamino hippurate (PAH) solution. AFV was quantitated by spectrophotometric assay of PAH concentration. Oligo- and polyhydramnios were defined as < 400 ml and > 1500 ml, respectively, for AFV (Queenan:Am J Obstet Gynecol:1972:114:34).. Regression values were 0.79 and 0.33 for AFV on AFI and on MAPD, respectively. True positive rates for oligohydramnios defined as AFI < 5cm or MAPD < 2 cm were 33% and 0%, respectively; false positive rates were 0%. True positive rates for polyhydramnios, defined as AFI > 20 cm and MAPD > 8cm were 100%; false positive rates were 20%. Overall predictive accuracies were 73%(AFI) and 58% (MAPD). AFI is superior to MAPD or "eyeball" for actual AFV estimation;both indices **overestimate** actual AFV at **both** of its extremes.

164 INTRA- AND INTER-OBSERVER VARIABILITY OF THE AMNIOTIC FLUID INDEX J. Bruner, A. Harrington*, M. Goodman*, A. Sarno, Dept. of OB/GYN, Vanderbilt Univ. Medical Center, Nashville, TN.

In an attempt to assess intra- and inter-observer variations in performance of the amniotic fluid index (AFI), 34 women in the third trimester with intact membranes were examined twice by each of 3 examiners. The AFI was measured once by each of the 3 examiners in order, then repeated by each examiner in the same order. Numerical displays on the video terminal screen were covered so that the examiners were blinded to actual measurement values. Results were recorded on hard copy for later data analysis. A senior investigator supervised all examinations in order to monitor consistency of technique. Overall, AFI measurements varied from about 15% within examiners to about 25% between examiners. Both between and within variation of absolute differences increased as the AFI value increased, while the percent difference decreased. For those AFI values <150 mm, SD was 9.85 mm, while SD of values >150 mm was 16.4 mm. If a discriminatory AFI value of $\leq$ 50 mm was used to identify oligohydramnios, a single value at the cutoff point (50 mm) could vary on average from 35 to 65 mm. If a value of $\geq$ 250 mm was used to identify polyhydramnios, a single value at the cutoff point (250 mm) could vary on average from 215 to 285 mm. Simply repeating the test by the same examiner for those values that fall in the discriminatory zone will decrease the variability on average to 40 to 60 mm (oligo), and to 225 to 275 mm (poly).

165 SONOGRAPHICALLY THICK PLACENTAS: INCREASED PERINATAL MORBIDITY AND MORTALITY. MP Dombrowski, HM Wolfe, AA Saleh, MI Evans, Dept of Ob/Gyn, Hutzel Hosp./Wayne State Univ., Detroit MI

In order to determine the incidence and significance of sonographically thick placentas, we reviewed the computerized records of 18,827 viable, singleton pregnancies. Of these, 116 (0.6%) had placentas which appeared subjectively thick and measured at least 4 cm by ultrasound. Thick placentas, initially diagnosed at a mean of 28.6±5.5 weeks (range = 15 to 38), were associated with shorter gestations (36.6±4.5 v 38.5±3.1 wks, $p < .0001$), decreased birth weights (2832±967 v 3070±718 g, $p < .05$), 31 (26.7%) had decreased amniotic fluid (AFV), 8 (6.9%) had oligo, 13 (11.2%) had increased AFV, and 6 (5.2%) had polyhydramnios. Odds ratios and 95% confidence limits (CL) for adverse pregnancy outcomes associated with thick placentas are listed below:

5-min Apgar < 7	6.7 (CL 4.0 to 11.3)
Abruptio placentae	2.9 (CL 1.1 to 8.1)
NICU admission	4.1 (CL 2.8 to 6.1)
Anomalies	8.4 (CL 4.9 to 14.4)
Perinatal mortality	12.0 (CL 7.5 to 19.4)
Total morbidity/ mortality	4.5 (CL 3.1 to 6.6)

Possible causes of the 116 thick placentas included: Rh isoimmunization = 6, diabetes = 11, abruptio = 4, but none with syphilis. Congenital anomalies, and not the above, were the leading cause of perinatal mortality. On the basis of these data, pregnancies with thick placentas are at risk for compromised perinatal outcome; sonographic evaluation should be peformed to rule out coexistent anomalies.

166 PERINATAL OUTCOME WITH SONOGRAPHICALLY THIN PLACENTAS. MP Dombrowski, AA Saleh, SM Berry, DB Cotton, Dept Ob/Gyn, Hutzel Hosp./Wayne State Univ., Detroit, MI

In order to determine the incidence and significance of sonographically thin placentas, we reviewed the computerized records of 18,937 viable, singleton pregnancies. Of these, 122 (0.6%) had subjectively thin placentas by ultrasound. The diagnosis of a thin placenta was initially made at a mean gestational age of 29.4±7.4 weeks (range = 8.6 to 40.1 weeks). Thin placentas were not associated with shorter gestations (38.6±3.0 v 38.5±3.1 wks), but 20.5% had polyhydramnios, 19.7% had increased amniotic fluid volume (AFV), 12.3% had decreased AFV, and 1.6% had oligohydramnios. Odds ratios and 95% confidence limits (CL) for thin placenta adverse pregnancy outcomes are listed below.

Weight < 10th %tile	2.4 (CL 1.5 to 3.8)
Abruptio placentae	2.0 (CL 0.6 to 6.5)
NICU admission	2.2 (CL 1.4 to 3.5)
Anomalies	3.6 (CL 1.7 to 7.4)
Perinatal mortality	3.4 (CL 1.7 to 7.1)
Total morbidity/ mortality	2.1 (CL 1.4 to 3.1)

Possible causes of the 8 perinatal mortalities with thin placentas included: esophageal atresia, sacrococcygeal teratoma with abruptio, anencephaly, placental hemorrhagic endovasculitis, thoracic dysplasia, abruptio, diaphragmatic hernia, and ventricular septal defect. In conclusion, pregnancies with thin placentas are at increased risk for perinatal morbidity and mortality; a careful sonographic evaluation should be performed to rule out coexistent fetal anomalies.

167 **SIMPLE GEOMETRIC RELATIONSHIPS BETWEEN AMNIOTIC FLUID INDEX AND AMNIOTIC FLUID VOLUME.** Robert A Brace[X]. Division of Perinatal Med, Dept of Reproductive Med, University of California, San Diego, CA 92093

The 4 quadrant amniotic fluid index (AFI) is used increasingly as an indicator of amniotic fluid volume (AFV) even though no established relationship exists between these two variables. The purpose of this study was to establish idealized relationships among AFI, AFV, and fetal size by treating the uterus and the fetus as either spheres or circular ellipsoids (len/dia=2). Using a computer, the weight of the fetus was varied from 0 to 3000 gm and the AFI from 20 to 300 mm. AFV (ml) was determined as the difference between uterine and fetal volumes. We found that the size of the fetus had dramatic effects on the relationship between the AFI and AFV. That is, as fetal weight increased, there were large shifts upward and to the left in the curve relating AFI (x) to AFV (y). For example, for AFI = 100 mm, AFV for a 3000 gm fetus (876 ml) was 26.1, 7.9, and 2.0 times that of a 10, 100, and 1000 gm fetus, resp. In addition, when the size of the fetus was near zero, there was a cubic relationship between AFI and AFV. As fetal weight increased, the relationship between AFI and AFV became progressively less curvilinear. For a fetal weight of 3000 gm, the relationship was nearly linear (AFV = 10.7 x AFI - 162, $r=0.998$, $p<0.00001$ for spheres and AFV = 14.6 x AFI -271, $r=0.996$, $p<0.00001$ for ellipsoids). These relationships help explain the near linear relationships between the AFI and AFV previously observed in animal and human studies. Thus, the relationship between the AFI and AFV is unique for each gestational age because of the strong dependence on fetal weight.

168 FETAL ILIAC BONE AND FEET MEASUREMENTS: NOMOGRAMS AND APPLICATION IN SKELETAL DYSPLASIAS. R. Jaffe, W. Meyer,[X] S. Warsof University of Illinois, Chicago, IL.

Skeletal dysplasias is a heterogenous group of disorders affecting the skeleton. With detection of disorders of bone growth a general diagnosis is often made in-utero because many anomalies have similar features. This study was performed to create nomograms for growth of iliac bone(IB), length and width of fetal feet as well as their growth relative to the femur. The study included 250 women. Correlation between gestational age (GA), femur length (FL), iliac length and feet measurements were established. The growth of IB and feet were found to correlate linearly with GA with a R^2 of 0.933 for IB, 0.828 for length and 0.820 for width of fetal foot. There was a linear relation between feet measurements and FL with a R^2 of 0.875 for length and 0.805 for width of foot. The IB is affected in many skeletal dysplasias and other anomalies whereas in some it is unaffected. The fetal feet measurements were shown to correlate well with both GA and FL. Both IB and feet measurements improved the diagnosis of skeletal dysplasias found in-utero. CONCLUSION: The addition of nomograms for IB and fetal feet measurements will enable improved recognition of specific anomalies and patient counseling.

169 COMPARISON OF DIFFERENT ULTRASOUND PARAMETERS IN THE PREDICTION OF SMALL-FOR-GESTATIONAL AGE (SGA) INFANTS.

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Many different ultrasonic parameters have been used to predict birthweight below the 10th percentile (SGA). Results have invariably been presented as sensitivity and positive predictive value; the latter being highly prevalence dependent. No previous reports have attempted to present summary statistics for each ultrasound variable for comparison. We have reviewed the literature from the past 15 years (72 studies) regarding the ultrasonic prediction of SGA. Studies were included if; a) the reported population was high risk; b) the outcome measure was a birthweight < 10th centile (using appropriate population charts); and c) the necessary information to construct a 2 x 2 table was reported. Only ultrasound variables reported in at least 2 different studies were included. For each variable a pooled sensitivity and common odds ratio with 95% confidence intervals is reported.

Results

U/sound parameter	Sensitivity	Common Odds Ratio	95%confidence interval
BPD<10th centile	63.7	5.83	(3.62 -9.37)
HC / AC >95th	47.6	3.34	(1.55 - 7.99)
FL / AC >23	49.	2.82	(1.71 - 4.66)
EFW < 10th	77.9	39.07	(28.9 - 52.7)
AC < 10th	84.4	18.38	(9.83 - 34.33)
Grade III Placenta	61.7	3.05	(1.7 - 5.25)

Conclusion: In a high risk population, AC has the highest sensitivity and EFW the highest common odds ratiofor the prediction of SGA. Comparable data for low risk populations and for the prediction of neonatal ponderal index will also be presented.

170 EVALUATION OF OBSTETRICAL ULTRASOUND AS A LABORATORY TEST AT FIRST VISIT. Browne PC, Hamer LH, Vroon DH[X], Clark WS[X] Department of Gynecology/Obstetrics, Emory University School of Medicine, Atlanta, GA

We compared routine obstetrical ultrasound to other standard laboratory tests performed at the first obstetrical visit in an indigent, inner-city patient population. We examined "normal" and "abnormal" values for blood type, rhesus group, antibody screen, rubella serology, syphilis serology, hepatitis serology, HIV serology, urine culture, GC test, chlamydia test and cervical cytology. A routine obstetrical ultrasound identified many clinically important findings such as fetal demise, multiple gestation, incorrect dating, fetal anomalies, etc. in more that half of patients studied. In our clinic population, routine obstetrical ultrasourd compares favorably with other recommended laboratory tests obtained at first visit.

ULTRASOUND		STANDARD LABORATORY TEST	
Incorrect Dates	28.6%	Rubella Non-immune	13.1%
Adnexal Masses	06.0%	Rhesus Negative	07.0%
Non-viable Pregnancy	04.9%	Abnormal Hemoglobin	06.1%
Uterine Anomalies	02.5%	Positive Drug Screen	04.7%
Abnormal Fluid Volume	02.2%	Positive Antibody Screen	01.4%
Placenta Previa	00.6%	HIV Positive	00.6%
Fetal Anomalies	00.6%	Hepatitis B Positive	00.5%

171 ADVERSE FETAL CARDIAC EFFECTS OF ORAL RITODRINE TOCOLYSIS. D.M. Friedman[X], J. Blackstone[X], I. Hoskins, Div. Pediatric Cardiology/Mat-Fetal Med, New York Univ. Med. Ctr. NY

The beta-sympathomimetic oral tocolytic, ritodrine (R), can cause maternal tachycardia and hypotension, and may cross the placenta. A new echo-Doppler technique was developed to explore fetal and placental R effects in 76 controls and 18 studies on stable oral R doses, at baseline and 30 min later. Data collected: maternal pulse and BP, fetal cerebral and umbilical Doppler waveforms, and FHR. A new index of fetal myocardial contractility, combined ventricular shortening fraction (CVSF), was derived from 2D directed M-mode. RESULTS: Maternal pulse and BP, FHR and fetal heart size, and all Dopplers were normal, without dose-response effects. In normals, CVSF fell with increasing gestational age (CVSF=-.27 EGA + 49, F=5.8, P≤.001, SEE=11). CVSF in R pts. was abnormally decreased, at either peak or trough, in 72% of cases. The mean CVSF in normals was 43 ± 5% but in R pts. was 31%. CONCLUSION: Premature labor and/or oral R is associated with reduced CVSF. Since there was no change in placental resistance, cerebral hypoxia, FHR, or heart size (preload), then low CVSF may be due to increased fetal systemic vascular resistance (BP) or decreased myocardial contractility.

172 SIGNIFICANCE OF THE ULTRASONOGRAPHIC DIAGNOSIS OF AMNIOTIC BAND IN RELATION TO FETAL OUTCOME AND MATERNAL COMPLICATIONS.

H. Wehbeh[X], M.D., A. Karimi[X], M.D., E. Ioannou[X], B.A., H. Minkoff, M.D. State University Health Science Center at Brooklyn, New York.

Introduction: In the routine practice of obstetrics, the ultra-sonographic diagnosis of an amniotic band is not rare and presents the physician and the patient with a counseling dilemma. Specifically, reports of limb disruptions and craniofacial anomalies raise major concerns. Materials: In an attempt to assess the prognostic significance of an ultrasonographic diagnosis of amniotic band, a retrospective review of ultrasonographic records at SUNY Health Science Center at Brooklyn and Kings County Hospital was performed. Between 1986 and 1991 25 cases of amniotic band were diagnosed. Cases were compared to 25 control patients who had ultrasound evaluations at the same gestational age (GA). Outcomes included anomalies (amniotic band syndrome), obstetrical complications (e.g. preterm labor, premature rupture of membranes (PROM), birth weight) and maternal factors that might predispose to bands (e.g. previous cesarean section, previous termination of pregnancy). Results: All cases had normal fetal anatomy and unrestricted fetal movement on the index sonogram (1 in the first trimester, 13 in the second and 11 in the third). No component of the amniotic band syndrome was found in the newborns, all of whom were liveborn. Sixteen (64%) had ultrasound follow up of which 9 revealed the disappearance of the amniotic band. No significant difference was found in fetal outcomes, maternal risk factors or complications between the two groups, except for preterm labor which was more common among cases. [Table 1]. Conclusion: This study suggests that the ultrasonographic diagnosis of an amniotic band in conjunction with sonographic findings of normal fetal anatomy and unrestricted fetal movement may carry minimal risk to the fetus and the mother. Preliminary evidence of an association with preterm labor needs to be confirmed.

Table 1. Obstetrical Outcome

	Case (n = 25) mean (SD)	Control (n=25) mean (SD)	P
Birth Wt (g)	2813 (680)	3045 (929)	.31
GA (wk)	37.4 (2.8)	37.4 (5.9)	.95
PROM	12% (.33)	16% (.37)	.95
Preterm Labor	20% (.41)	0.0 (0.0)	.02

173 **CLINICAL SIGNIFICANCE OF UTERINE LEIOMYOMATA IN PREGNANCY.** A. Ghidini[X], P. Vergani[X], N. Strobelt[X], N. Roncaglia[X], A. Spelta[X], A. Locatelli[X]. Mt. Sinai School of Medicine, New York, NY, St. Gerardo Hospital, Monza, Italy.

The clinical significance of uterine myomas in pregnancy has only been examined in small retrospective studies, leading to conflicting results. From Jan. 1983 to Jan. 1988 we followed prospectively 209 pregnant patients with sonographically identified myomas and 7523 pregnant women, without myomas. Site, number and location of myomas were documented. Among patients with fibroids, 12 had elective abortions performed, one had an ectopic pregnancy and 16 delivered elsewhere, leaving 180 cases for analysis. Spontaneous abortions (SAb's) were not more common in cases compared with controls (7.8% vs 8.3%, p=NS). SAb's were not related to myoma size, or location; however SAb's were more common with solitary compared with multiple myomas (5.5% vs 17%, p=0.02). Preterm deliveries were not more frequent in cases than in controls (9.6% vs. 9.7%). Cesarean sections were more common in cases than in controls (23% vs 14%, p<0.001), in lower uterine segment compared with fundal myomas (38% vs 17%, p<0.01), and when the mean myoma diameter was > 5cm (35% vs 16%, p<0.01). There were no increased occurrences of placental abruptio (1.2% vs 0.4%), fetal growth retardation < 5th percentile (3.6% vs 4.5%), preterm membrane rupture (2.4% vs 4.0%), post-partum hemorrhage > 500 cc (16.5% vs 17.6%), or post-partum endometritis (0.8% vs 0.9%), in cases compared with controls. However there was an increased prevalence of placenta previa in cases compared with controls (3.6% vs 0.6%, p<0.01). SUMMARY: Uterine myomas were found to be associated with an increased occurrence of cesarean sections and placenta previa, but not of SAb or preterm delivery.

174 **WHAT IS THE PREDICTIVE VALUE OF A FOUR-CHAMBER VIEW OF THE FETAL HEART IN THE PRENATAL DIAGNOSIS OF CONGENITAL HEART DEFECTS?** A. Ghidini[X], P. Vergani[X], S. Mariani[X], R. Schiavina[X], I. Ciarla[X], A. Spelta[X], N. Strobelt[X]. Mt. Sinai School of Medicine, New York, NY and St. Gerardo Hospital, Monza, Italy.

In January 1987 we introduced the four-chamber fetal heart view to screen for congenital heart defects (CHDs) during all prenatal ultrasound examinations. We now compare the detection rate for CHD during the subsequent three years (1987-89) to that during the two preceding years (1985-86). Routine ultrasound examinations were performed on 9016 women during the period 1985-89. All patients were followed through delivery or termination of the pregnancy, and neonatal clinical or autopsy confirmation of prenatal findings were available on all cases. The four-chamber view was considered abnormal if any of the following findings were detected: ventricular disproportion, myocardial hypertrophy, dilation or hypoplasia of any cardiac chamber, septal defect, or atrio-ventricular valve deformity. The overall prevalence of CHDs was 0.53% (48/9016). A four-chamber view of the fetal heart was obtained in 95% of cases. During the years 1985-86, 16 neonates with CHDs were identified, 7 of which were prenatally diagnosed (sensitivity 43%). During the period 1987-89, 32 cases of CHDs occurred, 26 of which were diagnosed antenatally (sensitivity = 81%, p = 0.01). No false positive diagnoses were made in either time-period, therefore the specificity was 100%. The four-chamber of the fetal heart is easily obtained, does not significantly increase the duration of a routine ultrasound examination and has an excellent sensitivity for the identification of CHDs.

175 **USE OF THE TRANSVERSE CEREBELLAR/ABDOMINAL CIRCUMFERENCE RATIO TO IDENTIFY GROWTH RETARDED FETUSES.** W.A. Campbell, A.M. Vintzileos, J.F. Rodis, G.W. Turner, J.F.X. Egan, D.Nardi.[X] University of Connecticut Health Center, Farmington, CT

SPO 1990-Abstract #519 reported that the transverse cerebellar/abdominal circumference ratio (TCD/AC) is gestational age independent and might be useful to diagnose intrauterine growth retardation (IUGR). We undertook this study to evaluate this. **Methods:** Patients were prospectively enrolled if, they had sure dates (1st trimester prenatal care and/or ultrasound ≤ 20 weeks), and their pregnancy was at risk for IUGR (eg. hypertension, drug abuse). At each ultrasound examination a transverse cerebellar diameter was obtained along with standard growth measurements. The TCD/AC was calculated for each examination. IUGR was defined as a birth weight (BW) ≤ the 10th %ile for gestational age. A TCD/AC was abnormal when > 15.9%. The examination to delivery interval was ≤ 14 days for all cases. **Results:** Eighty-seven (87) patients were analyzed. Based on BW, 48/87 (55%) neonates had IUGR. Comparing IUGR and non-IUGR groups, there was no significant difference in the mean gestational age at delivery, or examination to delivery interval (4 days). Growth measurements were significantly smaller in the IUGR cases (p< .05). The exception was the TCD measurement, which was not significantly different between the groups (p=.2). The mean TCD/AC ratio was 16.6% for the IUGR cases; significantly larger than non-IUGR cases (14.8%- p <.05). There were 14/48 (29%) IUGR cases missed by the TCD/AC; 57% of these cases had a BW < 3rd %ile. The TCD/AC had a sensitivity of 71%, specificity 77%, positive predictive value 79%, negative predictive value 68%. **Summary:** The TCD/AC ratio can be a useful adjunct for evaluation of fetuses at risk for IUGR. If BW is < 3rd%ile this ratio may be normal.

176 TRANSVERSE CEREBELLAR DIAMETER (TCD) IN TWIN GESTATIONS. T Shimizu[X], S Gaudette[X], C Nimrod, Division of Perinatology, Dept. of Ob/Gyn, Ottawa General Hospital, Ottawa, Canada.

Fetal biometric measurements are accepted to be useful for assessing intrauterine fetal growth in twin pairs. Although TCD is reported to be unaffected by IUGR in singleton pregnancy, there has not been any study on TCD in twin pregnancy. The present study compared TCD measurements in singletons with those in twins and also the effect of the chorionicity and discordancy on TCD growth. TCD was measured in both 94 fetuses of 47 normal concordant twin pairs and normal 329 singleton fetuses between 15 and 36 weeks. There was no significant difference in TCD measurements between normal singleton and twin gestations. TCD is unaffected by the chorionicity in normal concordant twin pairs. Mean % intrapair differences in BPD, TCD, HC, AC, FL, and estimated fetal weight were compared between concordant (n = 13) and discordant (n = 11) twin pairs. Only TCD and HC did not show significant differences (P = 0.801); P = 0.095). This study suggests that TCD is not impaired in discordant twin pairs and singleton normograms may be useful in all types of twin growth assessment.

177 TRANSCEREBELLAR DIAMETER IN TWIN GESTATIONS.

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Often there is a size discordance between the twins. This can be due to primary factors such as genetic differences or secondary to transfusion syndrome or intrauterine growth retardation. Previous studies using standard ultrasonographic biometry of biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL) have begun to establish normative growth curves. Importantly however, the use of these measures often results in significant differences in the gestational age (GA) estimates for co-twins. This is especially troublesome when ultrasongographic examinations are performed late in the gestation. Therefore, in these circumstances the clinician is hampered in attempts to determine the precise GA. In order to address the problem of different BPD's in twins we sought to use another measure which would have good correlation with GA and which would be more consistent between co-twins. We performed a prospective, cross sectional ultrasound examination on 43 well dated, uncomplicated twin gestations between 18 to 34 gestational weeks. The examination included measurements of transcerebellar diameter (TCD), BPD, AC and FL. A statistically significant linear relationship was found between TCD and GA (R^2=0.91; P<0.0000) and a curvilinear relationship between BPD and GA (R^2=0.94; P<0.0000) when the average TCD and BPD between co-twins were used for analysis. GA was derived from either BPD using Hadlock formula or TCD using Goldstein's formula. When these calculated GA's were compared with the known GA's, there was a significant difference in GA estimates derived from the BPD and TCD (P=0.0126). The average difference in GA between co-twins using TCD was 4.4 days versus 8.9 days using BPD. In conclusion TCD is a useful measurement to estimate the GA more accurately when there is a difference in BPD between the co-twins.

178 ULTRASOUND PREDICTORS OF FETAL MACROSOMIA AND BODY COMPOSITION IN INFANTS OF DIABETIC MOTHERS

RJ Kehl,PM Catalano,MA Krew, S Amini[X],A Thomas[X],LI Mann
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The purpose of this study was to prospectively analyze which ultrasound(US) parameters are most predictive of fetal macrosomia in infants of diabetic mothers(IDM);and to estimate whether fetal fat or lean body mass was increased in macrosomic(MAC)versus nonmacrosomic(NMAC) IDM and whether this could be determined using US. 34 women with gestational diabetes (n=25) and insulin dependent (n=9) women were prospectively examined in the third trimester. US measurements include BPD HC,FL, abdominal circumference (AC),transcerebellar diameter,kidney and liver length,SC abdomen and thigh fat.Each subject had at least 3 US's performed a minimum of 3 weeks apart.Each neonate had skinfold measurements and total body electrical conductivity (TOBEC) to estimate body composition.MAC was defined as birthweight>90% and NMAC was defined as <90% for gestational age(EGA).Twelve(38%) IDM were MAC and 22(62%) were NMAC and EGA at delivery was similar,MAC 37.6±1.4 NMAC 38.1±1.4wks p=.30.The overall mean growth rate of US measurements were compared using a Wilcoxon rank sum.There was a significant increase in AC,(mean±SD)MAC 12.2±2.0 NMAC 10.0±2.3mm/wk p=.02,SC abdomen fat,MAC 0.41±0.15 NMAC 0.22±0.16mm/wk p=.006,thigh fat,MAC 0.35±0.01 NMAC 0.19±0.18mm/wk p=.011,and liver length MAC 3.1±1.2 NMAC 1.9±0.92mm/wk P=.01 in the MAC as compared with the NMAC US measurements. In addition to birthweight,MAC 3874±334 NMAC 3070±420 p=.0001,MAC infants also had significantly greater skinfold measurements,MAC 12.5±2.3 NMAC 9.4±1.8 p=.0001,% body fat MAC 17.2±4.1 NMAC 10.8±4.2 p=.0002,and lean body mass,MAC 3215±146 NMAC 2726±345 p=.0001.Our results support previous findings that AC growth is predictive of MAC in IDM.Furthermore, the increase in AC appears to be secondary to an increase in both fetal fat (SC fat) and lean body mass (liver).Supported by NIH RR-00210 and 22965.

179 **A NEW ALGORITHM FOR RISK ASSESSMENT OF DIABETES-ASSOCIATED COMPLICATIONS DURING PREGNANCY:** E.A. Reece, G. Francis[X], Z. Hagay[X], Departments of Ob/Gyn at Temple University School of Medicine, Philadelphia, PA and Yale University School of Medicine, New Haven, CT

Classification schemes have failed to provide measurable means for prospective risk assessment of diabetes-associated complications making periconceptional counseling vague and imprecise. This study was undertaken to create mathematical models using both pre-pregnancy and intra-pregnancy conditions to quantitatively predict a patient's relative risk for adverse maternal and fetal outcome. The study population included 361 gestational diabetics (GDM), 205 uncomplicated pre-gestational diabetics (P-GDM), 82 complicated P-GDM, and 150 controls. Multivariate analysis was used to determine a patient's overall relative risk for a given outcome. A model was derived based on whether the variable was dichotomous or continuous. All the individual β values were entered into an equation: For dichotomous variables, the overall RR = $e^{(b_1 X_1 + b_2 X_2 + \ldots)}$. For continuous variables, the predicted outcome = $(I + b_1X_1 + b_2X_2 + \ldots)$ where I is a constant representing the intercept calculated during the multivariate analysis; b_1 = beta value for first independent variable; X_1 = actual value for first independent variable in model. For example, the relative risk for fetal distress can be assessed prior to pregnancy or during pregnancy as follows:

RISK FOR FETAL DISTRESS

Model I (Pre-Pregnancy Conditions)	Model II (Intra-Pregnancy Conditions)	Model III (Pre- and Intra-Pregnancy Cond.)
duration: RR=1.06/yr	Proteinuria: RR=1.92	duration: RR=1.06/yr
HBP: RR=1.8	Late HTN: RR=2.2	Proteinuria: RR=1.7
Gravidity: RR=1.18		Gravidity: RR=0.8

CONCLUSION: This new algorithm provides for the first time a measurable and thus useful means of estimating possible fetal and/or maternal complications that may arise during the antepartum or peripartum periods.

180 **THE POSITIVE PREDICTIVE VALUE OF A SONOGRAPHIC DIAGNOSIS OF FETAL MACROSOMIA.** Raphael N. Pollack[X], Michael Y. Divon, Dept. Ob/Gyn, The Albert Einstein College of Medicine, Bronx, NY.

The positive predictive value (PPV) of the sonographic diagnosis of fetal macrosomia was prospectively evaluated in 519 pregnancies of > 41 weeks gestation examined within 1 week of delivery. Estimated fetal weight (EFW) was obtained using measurements of abdominal circumference and femur length and the table of Hadlock et al. The PPV of varying sonographic EFWs in predicting birthweights of ≥4000 and ≥4500 grams, respectively, is shown:

Ultrasound EFW (Gms.)	Probability of Actual BW ≥4000 Gms.	≥4500 Gms.
3500	36%	7%
3600	40%	8%
3700	44%	9%
3800	49%	11%
3900	56%	14%
4000	66%	17%
4100	70%	19%
4200	71%	20%
4300	75%	21%
4400	80%	26%
4500	83%	29%
4600	80%	20%
4700	86%	29%

We conclude that the PPV of a sonographic diagnosis of macrosomia increases with increasing EFW. 75% of fetuses with an EFW of 4300 grams or larger will indeed be macrosomic by birthweight criteria.

181 "FETAL GROWTH CHARTS": COMPARISON OF CROSS SECTIONAL ULTRASOUND EXAMINATIONS WITH BIRTHWEIGHT. IM Bernstein, MC Meyer, GM Simmons, EL Capeless. Dept. Ob/Gyn, Univ. Vermont, Burlington, VT.

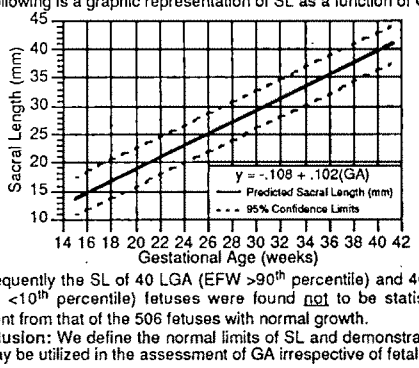
We examined the hypothesis that fetal growth curves derived from birthweight data underrepresent normal fetal weight in preterm gestation due to a high incidence of growth deficiency in premature newborns. We compared growth curves created from birthweight data with curves generated from ultrasound examinations collected cross-sectionally. We matched 350 ultrasound examinations with 350 newborn weights. Groups were evenly distributed by gestational age between 26 and 39 weeks gestation (25/wk). Ultrasound examinations were performed for size/dates discrepancy. Menstrual dating was confirmed by early ultrasound or index examination BPD. Regression lines for the growth curves were different ($p<0.001$). Between 26 and 35 weeks the ultrasound derived regression predicted higher fetal weights ($p<0.05$). We conclude that for preterm infants (<35 weeks) estimation of percentile rank for growth will differ between birthweight and ultrasound derived growth curves. Sonographically derived "fetal growth charts" provide an improved standard when characterizing ultrasound estimates of fetal weight.

182 FETAL SACRAL LENGTH IN THE ASSESSMENT OF GESTATIONAL AGE. David M. Sherer, Jacques S. Abramowicz, Mark A. Plessinger*, James R. Woods, Jr. University of Rochester, Rochester, NY.

The fetal sacrum is a consistent sonographically identifiable structure. In a prospective cross-sectional study of 506 singleton fetuses between 15-41 weeks, with normal growth and no structural anomalies, the sacral length (SL) ranged between 15-43 mm. Models to predict SL based on gestational age (GA), biparietal diameter (BPD), head circumference (HC) and femur length (FL) and 95% confidence limits were derived by least squares regression analysis. The SL was analyzed as the dependent variable paired with the GA, BPD, HC and FL as the independent variables. Scatterplots of the data for each model along with the standard error of the estimate (SEE), coefficient of determination (R^2) and adj. R^2 demonstrated excellent correlation:

SL/GA: SEE=0.1707, R^2=95.9%, Adj. R^2=95.9%
SL/BPD: SEE=0.2082, R^2=93.9%, Adj. R^2=93.8%
SL/HC: SEE=0.2048, R^2=94.1%, Adj. R^2=94.0%
SL/FL: SEE=0.2352, R^2=92.2%, Adj. R^2=92.1%

The following is a graphic representation of SL as a function of GA:



Subsequently the SL of 40 LGA (EFW >90th percentile) and 40 SGA (EFW <10th percentile) fetuses were found not to be statistically different from that of the 506 fetuses with normal growth.

Conclusion: We define the normal limits of SL and demonstrate that SL may be utilized in the assessment of GA irrespective of fetal size.

183 UNEXPLAINED MIDTRIMESTER POLYHYDRAMNIOS: SONOGRAPHIC FOLLOW-UP AND PERINATAL SIGNIFICANCE

Jacques S. Abramowicz, MD, David M. Sherer, MD, J. Christopher Glantz, MD, James R. Woods, MD. University of Rochester, Rochester, NY.

Third trimester polyhydramnios has been associated with suboptimal perinatal outcome such as higher incidence of preterm labor, pre-eclampsia,placental abruption, and fetal anomalies. The significance of midtrimester polyhydramnios is less clear. We examined the outcomes of 47 singleton gestations from 16 to 27 weeks gestation with unexplained polyhydramnios but with no known structural anomalies or evidence of maternal diabetes. Polyhydramnios was defined as mild-moderate if the greatest vertical fluid pocket visualized on ultrasound measured > 6cm and severe if it measured >10cm. A group of 87 gestations with normal amniotic fluid and identical inclusion criteria constituted the control group.

Results: Demographic and obstetrical data were similar in both groups. In the study group, 44 patients (94%) were diagnosed as mild-moderate and 3 as severe polyhydramnios. Follow-up scans were performed on 40 patients. Among these, polyhydramnios had resolved in 30 (75 %). Of those with mild-moderate polyhydramnios who were rescanned, it had resolved in 74%. In severe polyhydramnios with subsequent scans, it resolved in all (n=2). Gestational age at delivery, mean birthweight, 5min Apgar scores, incidence of pre-eclampsia, and placental abruption were similar in both groups. In the study group, 3 infants were subsequently diagnosed as having an anomaly: two cases of trisomy 21 and a third with unilateral multicystic dysplastic kidney. No anomalies were demonstrated in the control group ($p<0.025$).

Conclusions: Resolution of unexplained midtrimester polyhydramnios is common. Further antenatal investigation, however, may be indicated, even in the absence of additional abnormal sonographic findings.

184 FOOT:LEG LENGTH AND FOOT:FEMUR LENGTH RATIO IN NONINVASIVE SCREENING FOR TRISOMY 21. Mark Paul Johnson[X], Mason Barr Jr.[2X], Marjorie C. Treadwell[X], Nelson B. Isada, Peter G. Pryde[X], David B. Cotton, Mark I. Evans. Dept. OB/GYN, Hutzel Hospital/Wayne State Univ., Detroit, MI., and Depts. OB/GYN, Pediatrics & Pathology, Univ. of Michigan, Ann Arbor, MI.

Ultrasound screening for trisomy 21 has had limited success. However, fetuses with T21 tend to be growth restricted and may have shortened limb lengths. In order to delineate the pattern of IUGR in T21, gravimetric measurements were collected from 436 fetal necropsies (gestational ages (GA) 100-200 days) from the Teratology Database at the Univ. of Michigan (391 morphologically normal and 45 karyotype confirmed T21 fetuses) Using the power equation analytical approach we have previously described, we confirmed that *foot length* and *leg length vs. GA* are linear relationships. We then generated a *foot to leg length ratio* and found it to be linear vs. GA in both normal and T21 populations. However, the generated regression curves were found to be significantly different between groups ($p<.0001$) with higher ratios noted in T21. Using a *foot to leg length ratio* of >0.44 to screen for T21, the following were calculated: sensitivity=0.69, specificity=0.91, +PV=0.61, and -PV=0.94. In this population, with a T21 prevalence of 17%, this measurement provides a high +PV and -PV for the identification of T21. We conclude that 1) *foot to leg length ratios vs. GA* is a linear relationship, and 2) regression curves are significantly different between T21 and normals. This finding now provides a rational anatomic basis for ultrasound screening for T21. Since completion of this analysis, and based on our observations that T21s have shortened upper leg lengths, we have begun a prospective study using *foot to femur lenth ratios* measured by ultrasound during routine prenatal screening. Preliminary data reflects the differences found in our *foot to leg length* necropsy studies and has encouraged our continued work to define the role of this measurement in prenatal screening for T21 in a low risk population.

185 **NECK CIRCUMFERENCE MEASUREMENTS IN SECOND TRIMESTER FETUSES WITH DOWN SYNDROME.** G.W. Turner, A. Vintzileos, D. Nardi[X], L. Feeney[X], W. Campbell, J. Rodis, University of Connecticut Health Center, Farmington, CT

Excessive nuchal skin thickening has been described as one of the features of Down syndrome (DS) during the second trimester as well as after birth. The purpose of this prospective study was to investigate the usefulness of fetal neck circumference (NC) during the second trimester to detect fetuses with DS. Single sonographic examinations of 132 patients referred for genetic amniocentesis between 12 and 25 weeks of gestation were used to generate the nomograms of fetal NC versus gestational age (GA), biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL). All fetuses were subsequently proven to be karyotypically normal. The fetal NC was determined by placing the transducer at a right angle to the cervical vertebrae so that the fetal neck was imaged at a plane in which the cross sectional area was the largest and as round as possible. From this view, the NC was indirectly calculated from two perpendicular diameters which were measured from outer to outer border. The mean intraobserver and interobserver variabilities in NC measurements were 6.1% (range: 1.3%-16.1%) and 6.8% (range: 0%-14.5%), respectively. Plotting of fetal NC measurements vs. GA, BPD, HC, AC and FL revealed linear relationships. The 5th, 50th and 95th percentiles were established. Subsequently, nine fetuses with DS were identified between 16 and 21 weeks of gestation among 1,186 fetuses (prevalence of DS in this population 1:132). Only 1 of the 9 fetuses had an abnormally increased NC (sensitivity 11.1%). The remaining 8 fetuses were equally distributed above and below the 50th percentile. The sensitivity, specificity, positive and negative predictive values of the fetal NC versus GA were 11% (1/9), 95% (1,119/1,177); 1.6% (1/59), and 99% (1,119/1,127), respectively. Our observations suggest that fetal NC measurement in the second trimester is not useful in the prenatal diagnosis of DS.

186 **CORRELATION OF UTERINE FUNDAL HEIGHT WITH ULTRASOUND IN TWIN GESTATIONS.** James FX. Egan, Anthony Vintzileos, Garry Turner, John Rodis, Winston Campbell, Edward Wolf, James Balducci, Luanna Lettieri, Judith Mead, Department Ob/Gyn, University of Connecticut Health Center, Farmington, CT

Due to the lack of fundal height nomograms in normal twin gestations, it has been a standard recommendation to use frequent ultrasound examinations in order to diagnose discordant fetal growth. The rationale of such a practice however, has not been established. The purpose of this prospective, cross sectional study of 152 twin pregnancies was to determine a nomogram for FH in normal twin gestations (n=132) and to see if FH can detect discordant (≥20%) growth in twins (n=20). After a scan FH measurements were obtained by both attending physician and fellow. Maternal age, gravity, parity, height and weight, gestational age (GA), fetal presentation, placentation, amniotic fluid volume, estimated fetal weight and % discordance were also recorded. Examinations were performed from 18 to 38 weeks (wks) GA. Results: Mean FH (±SD) was 5.9 (±2.9) cm greater than GA in wks; in concordant twins and 6.5 (±5.2) cm for discordant twins (p=NS). If the maternal weight was <200 lbs the FH averaged 5.4 (±2.9) cm more than GA in wks, while if the maternal weight was > 200 lbs the fundal height averaged 8.1 (±2.7) cm more. The mean (±SD) interobserver difference in FH measurements was 1.6 (±1.2) cm. FH did not adequately detect discordant growth in twins. The sensitivity was 12%, specificity 75%, positive predictive value 76%, negative predictive value 11%. It is concluded that FH measurement cannot be used as a screening clinical test for discordant fetal growth. Because of the low sensitivity (12%) our study supports that routine monthly ultrasound monitoring is necessary for the diagnosis and follow up of discordant fetal growth.

187 **FETAL STOMACH MEASUREMENTS ARE NOT REPRODUCIBLE.** E. Z. Zimmer[X], C. R. Chao[X], G. Abramovich[X], I. E. Timor-Tritsch, Dept. of Ob/Gyn, Columbia University, New York, NY

Several groups have published tables of normal dimensions of the fetal stomach. We hypothesized that because stomach filling and emptying is a dynamic process, measurement of stomach dimensions might be subject to error due to changes in stomach volume over relatively short periods of time. We measured stomach anteroposterior, transverse, and longitudinal dimensions at the beginning and end of a twenty minute period in 39 fetuses. To provide a standard for comparison, the same individual also serially measured biparietal diameter (BPD) twice at the same interval in 30 fetuses. The percent change between each of the paired serial measurements was then calculated and the mean percent changes compared between the stomach and BPD measurements: (mean ± SEM, Mann-Whitney-U test)

	Mean Percent Change	Significance Compared to BPD
Longitudinal	13.5 ± 2.0	p < 0.0001
Anteroposterior	17.7 ± 2.6	p < 0.0001
Transverse	15.1 ± 1.7	p < 0.0001
Volume	35.2 ± 4.7	p < 0.0001
BPD	1.2 ± 0.2	----

Because stomach measurements are subject to large variations in the same individual due to the dynamic nature of stomach dimensions and filling, we suggest that caution be exercised in the use of single measurements of stomach dimensions for the assessment of gestational age or the diagnosis of fetal abnormalities.

188 **DISTRIBUTION OF FETAL WEIGHT IN THE PRESENCE OF IDIOPATHIC POLYHYDRAMNIOS.** Asrat T, Thomas S, Towers CV, Nageotte MP, Major CA, Women's Memorial Hospital, Long Beach, CA, University of California, Irvine, CA.

We conducted a study to investigate the relationship of idiopathic polyhydramnios diagnosed in the third trimester and the incidence of fetal macrosomia. Between 9/88 and 12/90, 1550 patients had over 5000 serial amniotic fluid volume determinations. Of these, 157 were diagnosed with polyhydramnios, defined as an AFI of ≥20cm. Patients with diabetes, multiple gestations and congenital anomalies were excluded, resulting in 94 study patients with idiopathic polyhydramnios. The birthweight distribution of these infants according to the California growth curves is outlined below.

Distribution of Birthweight

Percentile	N(%)
<10th	17(7.5)
10th-50th	33(35.1)
51st-89th	44(46.8)
>90th	10(10.6)
TOTAL	94(100)

CONCLUSION: Only 10 infants, 10.6%, had birthweights >90th percentile, which reflects the normal distribution in California. The birthweight of infants born to mothers with idiopathic polyhydramnios is normally distributed. Unlike, in pregnancies complicated by diabetes, idiopathic polyhydramnios does not seem to correlate with fetal macrosomia.

189 RECOGNITION OF THE LARGE FOR GESTATIONAL AGE (LGA) FETUS USING GESTATIONAL AGE INSENSITIVE PARAMETERS. Joseph M. Miller, Jr., LSU Medical Center, New Orleans, LA.

Accelerated abdominal circumference (AC) growth occurs in the LGA fetus of diabetics and may allow improved recognition. The utility of the AC growth rate (AGR) and the ratio of the femur length (FL) to AC were evaluated in 35 diabetic and 52 nondiabetic patients, scanned after the 31st week on ≥2 occasions at least 2 weeks apart. The initial and final scans were analyzed. Birthweight (BW) tables of Brenner defined the median BW and LGA (>90%). AGR correlated with LGA status in diabetics but not in nondiabetics. FL/AC from the last study was strongly associated with LGA status in both groups. Both AGR and FL/AC correlated with relative BW (RBW) = BW÷median BW for GA, r=.408 and −.675, respectively, and may explain why these indices work better for larger LGA newborns of diabetics (RBW=1.37±.17) than nondiabetics (RBW=1.25±.08), p=.03. FL/AC may be more useful than AGR, particularly in diabetics

		Diabetics			Nondiabetics		
		LGA	NonLGA	p	LGA	NonLGA	p
AGR	≥1.2	10	4	.004	5	6	.100
cm/wk	<1.2	4	17		7	34	
FL/AC	<.21	13	4	.000	7	5	.003
	≥.21	1	17		5	35	

190 TRANSVERSE CEREBELLAR DIAMETER/ABDOMINAL CIRCUMFERENCE RATIO IN PREGNANCY: A NOMOGRAM W. Meyer,[X] D. Gauthier,[X] S. Warsof, A. Bieniarz University of Illinois at Chicago, Chicago, IL

In fetal growth disturbances, cerebellar growth has been shown to remain constant while abdominal circumference may vary considerably. To determine whether a relationship exists between fetal transverse cerebellar diameter (TCD) and abdominal circumference (AC), 138 patients with well dated pregnancies between 14-42 weeks were evaluated in a cross-sectional study. All pregnancies were normal, with no maternal or fetal factors which are associated with abnormal fetal growth. The TCD, AC and TCD/AC ratio were calculated for each patient and correlated to gestational age with linear regression analysis. RESULTS. Excellent correlation exists between TCD and gestational age (r=0.97) as well as TCD and AC (r=0.98). TCD/AC was normally distributed with a mean of 13.65 +/- 0.88%, (median=13.64%, mode=13.61%). The 5th and 95th percentiles were 12.27 and 15.28% respectively. The TCD/AC ratio remained constant throughout pregnancy when compared to gestational age (r=0.03). CONCLUSION: THE TCD/AC RATIO IS A STABLE FETAL BIOMETRIC PARAMETER WHICH IS INDEPENDENT OF GESTATIONAL AGE. THIS RATIO MAY BE USEFUL IN THE PRENATAL DETECTION OF SOMATIC FETAL GROWTH ABNORMALITIES.

191 FETUSES WITH DOWN'S SYNDROME HAVE DISPROPORTIONATELY SHORTENED FRONTAL LOBE DIMENSIONS ON ULTRASOUND. Bahado-Singh R, Wyse L,* Dorr MA,* Copel JA, and Hobbins JC. Department of OB/GYN, Yale University School of Medicine, New Haven, CT.

Shortened occipital frontal diameter (due to short frontal lobe) occurs in Down syndrome (DS) postnatally. We assessed frontal lobe size in mid-trimester DS fetuses. Frontal lobe length (FLL) and frontal lobe-cavum septum pellucidum (FLL-CSP) were measured from inner table to anterior and posterior aspect of cavum, respectively. Fronto-thalamic distance (FTD) from inner table to posterior thalamus and FTD/BPD were obtained. Nomograms were generated for 125 normals (15-21 weeks) and compared to 19 DS fetuses. FTD and FTD/BPD proved the most useful parameters. When expressed as multiples of the median to eliminate variation due to gestational age, significant shortening in mean FTD in DS fetuses compared to normals ($p<0.0019$) and FTD/BPD ($p<0.0177$) was seen. In the DS group, 32% had FTD <10th percentile. If an observed-to-expected FTD ratio of 0.84 is used as a screening test for DS, sensitivity 21.1%, specificity 95.2%, and positive predictive value 0.6 would be obtained in a population with 1:250 risk of DS. **Conclusion: Frontal lobe dimension is significantly shortened in DS fetuses. Prospective evaluation of this finding is planned.**

192 **AN EVALUATION OF RESIDENT USE OF LABOR AND DELIVERY ULTRASOUND: VALUE AND LIABILITY.** L. Coultrip, C. Ludowese[X], L. Hawkins[X], V. Lupo. Hennepin Medical Center, Minneapolis, MN.

In this ongoing study, ultrasound (US) use by obstetric residents assigned to Labor and Delivery is prospectively monitored to determine the usefulness and limitations of US performed by second year residents without formal US training. Of 1225 patients evaluated on L&D for acute problems and 480 service deliveries during a four month interval, 191 US examinations have been documented. Indications included amniocentesis (15%), placentation (13%), EFW (10%) and other (15%). The most common indication for a scan is dating (47%) in the absence of prenatal care. 55% of these patients presented at 28 weeks or less. 95% of GA estimates were deemed accurate when confirmed by subsequent scan by trained technicians or newborn exam. Using BPD/AC, 19% of EFWs fell within 5% and 48% within 10% of fetal delivery weight within 48 hours of scan (N=30) compared to 27% and 58% respectively for FL/AC. Missed diagnoses included a term twin gestation and a gastroschisis. In an indigent population presenting with erratic prenatal care, L&D scanning affords an opportunity to accurately date the pregnancy in a population in which 35% would otherwise be poorly dated.

193 OBSERVER VARIABILITY IN SONOGRAPHIC ASSESSMENT OF CERVICAL LENGTH

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Ultrasound evaluation of the cervix has been proposed as an objective means of assessing cervical change during gestation. Several studies have tried to correlate sonographic changes in cervical length with the risk of cervical incompetence and preterm labor. The present study was designed to test the variablity of cervical sonographic measurements by independent observers. Thirteen patients in the first trimester were evaluated by two clinicians who each performed two measurements of cervical length determined with a 7.5 mHz vaginal probe. The length of the cervix was measured from internal os to external os. For each enrolled patient, there was no significant difference in cervical length measurement when assessed by the same observer. For observer A, the mean ± SD for the two cervical length measurements were 2.67 ± 0.59 and 2.76 ± 0.56 cm (p = 0.23). For observer B, these measurements were 2.58 ± 0.56 vs 2.59 ± 0.54 cm (p = 0.91). The mean differences in cervical length / patient for observers A and B were 1.69 ± 1.6 cm and 4.15 ± 2.5 cm, respectively. Although mean cervical length was consistent for the same observer, there was a significant interobserver difference noted for each patient (2.71 ± 0.57 vs 2.59 ± 0.54 cm, p=0.001). We conclude that there is a significant difference beween examiners when measuring cervical length with vaginal ultrasound. Measurements were consistent, however, when performed by the same observer. If sonogrqaphic evaluation is to be used as an objective way of assessing cervical length in pregnancy, serial measurements must be performed by the same individual.

194 THE CONSEQUENCE OF ULTRASOUND DETECTION OF LATE SECOND AND THIRD TRIMESTER, MILD TO MODERATELY SEVERE FETAL URINARY TRACT DILATATIONS.

Ignatia B. Van den Veyver, M.D.[X], Jozef S. Venderheyden, M.D.[X], Luc A. Meeuwis, M.D.[X], Christine T. Vandeputte, M.D.[X], and Julien M. Norga, M.D.[X]; Depts. of Ob/Gyn and Ped.; St. Augustinushospital; Wilrijk, Belgium.

30 - 50% of the anomalies detected by routine antenatal ultrasound (US) originate in the fetal urinary tract (FUT). The interpretation, management, and outcome of these is still uncertain. **Purpose:** To investigate the frequency of FUT dilatations during the late second and third trimester; to correlate antenatal US with postnatal findings; to establish a followup protocol. **Materials and Methods:** 3 US/pregnancy were performed in 2000 patients with an Aloka 256 and a 650 SSD real-time US scanner (3.5 MHz). Dilatations were classified as mild (pelvis only), moderate (dilated calyces), or severe (↓ renal cortex). Antenatal followup: conservative when unilateral with normal amniotic fluid; delivery when bilateral with ↓ amniotic fluid and fetal lung maturity. Neonatal followup: US scan after 72 hours, urine culture in first week. Followup at 3 months when mild or normal. IVP, voiding cystography, and treatment when abnormal. **Results:** FUT dilatation in 1.45% (29/2000 pts); mild-65.5%; moderate-24.1%; severe-10.3%. Of the mild cases, 50% were normal at birth; 68.4% at 3 months; 20.6% of children had surgery done before 1 year of age or before symptoms occurred. **Conclusions:** Dilatation of the FUT is common on antenatal US; if mild limited followup is required. Early treatmert or surgery before symptoms can be offered after antenatal diagnosis.

195 SONOGRAPHIC RENAL PELVIS SIZE IN NORMAL AND DOWN SYNDROME FETUSES.

Kirk JS, Uckele JE[X], McNeil L[X], Rice M[X], Riggs T[X], Comstock CH, and Lee W. Div. of Fetal Imaging, Dept. OB-GYN and Dept. Pediatrics, William Beaumont Hospital, Royal Oak, MI.

Objectives: 1) To determine the size of renal pelves in normal fetuses throughout gestation. 2) To determine if renal pelvis size in Down syndrome fetuses differed from normal fetuses. Methods: From 9/1/90 to 6/14/91, an antero-posterior diameter of at least one renal pelvis was measured prospectively in fetuses when the fetal bladder was not distended. Only babies born at our hospital to mothers with a history of regular menses were included in the normal group. All newborn charts and karyotype results were reviewed for this group. There were 604 normal fetuses after anomalies and abnormal karyotypes were excluded. During the study period, 9 fetuses with Down syndrome had renal pelves measured. Retrospective review of videotaped scans of Down syndrome fetuses from the last 5 years added 25 Down syndrome fetuses with adequate views for measurement (total = 35). Results: In the normal fetuses, the renal pelvis diameter increased linearly with gestational age ($y = 0.01x + 2.78$, $r = 0.435$). Using the 95th percentile level as the upper limit of normal, abnormally full renal pelves occur at $\geq$ 4 mm for < 17 weeks, $\geq$ 5 mm for 17 to 30 6/7 weeks, and $\geq$ 6 mm for 31 weeks and over. Only 4 of the 35 Down syndrome fetuses had at least one abnormal renal pelvis diameter (binomial distribution p = 0.27) Conclusion: Renal pelvis size in Down syndrome fetuses did not differ significantly from normal fetuses.

196 **AURICULAR MID-CALVARIUM MEASUREMENT: THE ANTENATAL DIAGNOSIS OF LOW SET EARS**

Mark T Cullen MD, Jaqueline Green RDMS*, Luis Sanchez-Ramos MD, John C. Hobbins MD. University of Florida, Jacksonville, FL. and Yale University, New Haven,CT.

Low set ears is a congenital anomaly that can be associated with genetic syndromes and aneuploidy. Prenatal identification would assist in antenatal counseling and permit the option of karyotype analysis. We present data on the ultrasound evaluation and antenatal identification of low set ears. The study was prospective and cross sectional. Two hundred and sixty five well dated patients were referred for ultrasound examinations throughout pregnancy. Patients were examined once. After a thorough anatomic survey, an auricular to mid-cranial (AMC) measurement was obtained. This measurement was made on a coronal plane of the head at the level the thallamus, from the mid calvarium to the inferior insertion of the external ear. A nomogram was generated from the 265 well dated normal pregnancies with 95% confidence intervals. Follow up examination of the newborn was accomplished in all cases. There was a strong correlation between gestational age and the AMC (R=.94). There were 5 fetuses with low set ears that underwent ultrasound examination and had an AMC measurement, 4 were predicted prenatally. Low set ears were diagnosed in all three trimesters. The earliest diagnosis was made at 12 week. **Conclusion**: Low set ears can be diagnosed with ultrasound.

197 **ADVERSE FETAL OUTCOME ASSOCIATED WITH VARIX OF THE FETAL INTRAABDOMINAL UMBILICAL VEIN.** DP Reisner, BS Mahony,[X] JP McGahan,[X] DA Nyberg,[X] Swedish Hosp. Med. Ctr., Seattle WA and Univ. California-Davis, Sacramento CA

Varix of the fetal intraabdominal umbilical vein (FIUV) is a rare finding of uncertain etiology. We followed 11 fetuses with FIUV varix detected with prenatal ultrasound (US). US indications included elevated MSAFP (N=5), family history of congenital abnormalities (N=2), vaginal bleeding (N=1), abdominal mass on outside US (N=1), size/dates discrepancy (N=1), and preterm labor (N=1). Maternal age ranged from 21-35 years. One fetus, born to a 35-year-old, had Trisomy-21. Seven of 11 fetuses were males. One pregnancy is ongoing.

- IUFD (1 w/ Trisomy-21) 4/10 (40%)
- Abnormal karyotype (4 not tested) 1/7 (14%)
- Elevated MSAFP (4 not tested) 5/7 (71%)

CLINICAL OUTCOMES (N=11)

- 2nd trimester dx w/ IUFD at 27-30 wks 4/6
- 3rd trimester dx w/ survival (1 premature w/ hydrops) 4/4
- Ongoing pregnancy 1

One neonate had IUGR. Three macerated fetuses precluded accurate weights. Three of 4 fetuses with IUFD underwent autopsy, which did not reveal cause of death. Second trimester detection of FIUV varix may be associated with increased fetal morbidity and mortality and warrants close fetal surveillance.

198 IMPROVEMENT OF UMBILICAL ARTERY DIASTOLIC FLOW PREDICTS BETTER NEONATAL OUTCOME. JG Bell[X], A Ludormirsky,[X] J Bottalico[X], S Weiner, Pennsylvania Hospital, Philadelphia, PA

Fetuses that show absent end diastolic flow in the umbilical artery have a high risk of developing IUGR and oligohydramnios, have increased stillborn rates, and have significant perinatal and neonatal morbidity and mortality. Using doppler flow measurement as a clinical parameter for delivery timing is still very controversial. 60 pregnancies with a diagnosis of absent diastolic flow were studied. Fetuses were followed by traditional well being testing(BPP,NST,fetal movement assessment) and delivery decisions based on these parameters or worsening maternal condition. In 11 of 60 cases(18%), diastolic flow improved as the pregnancy progressed and gestation was prolonged with improved outcome. Results:

	Improvement	No Improvement
Oligohydramnios	3/11(27%)	21/49(43%)
IUGR by US(< 10%)	5/11(45%)	29/49(59%)
Dx to Delivery (days)	49(range 9-121)	11(range 0-57)
Stillborn	1/11(9%)	6/49(12%)
Gest. at Deliv(wks)	32.3(range28-37.6)	29.3(range 21-35)
Avg. Birthwt.(gms)	1418(range499-2400)	964(range280-1879)
Neonatal Death	1/11(9%)	12/49(25%)
Avg.# days in nursery	34(range 5-86)	69(range 25-186)
Survivors	9/11(82%)	30/49(61%)

Conclusion: The data emphasize the possibility of umbilical artery diastolic flow improvement in a group of fetuses that seemed to be in a decompensated state.

199 FETAL DESCENDING AORTA (DA) DOPPLER IN RESPONSE TO MATERNAL INGESTION OF LOW DOSE ASPIRIN (ASA). J.C. Veille, R. Hanson, L. Henderson, M. Swain, M. Sivakoff, Dept. of Ob/Gyn, Bowman Gray School of Medicine, Winston-Salem, NC and Case Western Reserve University, Cleveland, OH.

The effect(s) of chronic maternal ingestion of low dose ASA (40-80 mg) on the fetal DA blood flow (DAF) has not been documented. Pulsed Doppler was used to study DAF in fetuses exposed to ASA and compared to a control group. This was done at 3 different gestational periods: Groups (GRP) I = 12=23; II = 24-32; III = 33-41 weeks. A total of 230 studies were done throughout gestation. Six Doppler waveform were analyzed and averaged. The size of the DA was determined during systole, using a frame grabber. Results are reported as X ± SD. ANOVA was used to determine significance.

GRP	$DAPFV_C$	$DAPFV_{ASA}$	$DATVI_N$	$DATVI_{ASA}$
I	45.0 ± 2	46.2 ± 3	5.6 ± 0.3	6.1 ± 0.6
II	59.0 ± 3	58.5 ± 2	7.8 ± 0.4	7.3 ± 0.4
III	63.4 ± 3	56.5 ± 5	7.8 ± 0.4	7.2 ± 0.6

GRP	$DA"SV"_N$	$DA"SV"_{ASA}$	$DA"O"_N$	$DA"O"_{ASA}$
I	0.47 ± .08	0.35 ± .07	58.4 ± 8.6	57.4 ± 12.2
II	1.21 ± .11	1.03 ± .09	166.4 ± 16.7	143.6 ± 11.9
III	1.97 ± .19	1.89 ± .36	259.0 ± 26.1	274.5 ± 50.2
p	0.0000	0.0000	0.0000	0.0000

Legend: DAPFV = Descending aorta peak flow velocity (cm/sec); DATVI = descending aorta time velocity integral; DASV = descending aorta "stroke volume" (ml); DAO = Descending aorta output (ml/min); C = Control; ASA = Aspirin. Results: (1) No significant differences were found in PFV and TVI with advancing GA and among the "C" and "ASA" group. (2) Both the "SV" and "O" significantly increased with GA. No difference in the "C" and "ASA" groups were found. Conclusions: Maternal ingestion of daily low dose **ASA DOES NOT** significantly affect DA flow velocity and output. (Supported by NIH Grant HL38296).

200 **EFFECT OF LOW DOSE ASPIRIN (ASA) ON HUMAN FETAL RENAL BLOOD FLOW (FRBF).** J.C. Veille, R. Hanson, L. Henderson, M. Swain, Dept. of Ob/Gyn, Bowman Gray School of Medicine, Winston-Salem, NC

Low dose ASA has been used to prevent certain obstetrical complications. The effect of ASA on the FRBF has not yet been studied. FRBF was determined using pulsed Doppler in two groups of patients. One group of patients was used as a control group, the other group was taking 40-80 mg of ASA daily from the 12th week onward. Analysis was done at two different gestational (i.e. ≤28 weeks and >29 weeks) age) to determine any effect of maturity on FRBF in the control and in the ASA groups. A total of 143 studies were done (94 in control and 49 in ASA group). Three to six waveforms/study were analyzed and averaged. Results are expressed as X and SD. ANOVA test for repeated measurements was used to detect statistical significance.

Groups	I (≤28 wks)	p	II (≥29 wks)	p
S/D ASA	7.33±3.1	NS	6.83±1.1	NS
S/D Control	8.02±2.2		5.88±2.5	
PFV ASA	33.65±9.6	NS	36.25±9.7*	<0.02
PFV Control	29.50±10.2		24.49±10.4	
TVI ASA	5.23±2.0	NS	6.27±2.4*	<0.02
TVI Control	5.01±1.8		4.29±1.6	

(Legends: S/D=systolic/diastolic ratio; PFV=peak flow velocity (cm/sec); TVI=time velocity integral; ASA=aspirin) Results: 1) The S/D waveform was not different between the two groups at the both gestational ages; 2) In the "early" gestational age group no difference was found in PFV and TVI between the two groups; 3) In the "more advanced" gestational age, PFV and TVI were significantly greater in the ASA group when compared to control. Conclusions: ASA was found to significantly affect the human fetal renal vascular bed at or after the 29th week. This may be through a direct or an indirect effect of a prolonged maternal ingestion of ASA on this vascular bed or other vascular beds like the ductus, the aorta, the ventricles or the systemic vasculature. (Supported by NIH Grant HL38296).

201 **FETAL INTRACARDIAL EFFECT OF MATERNAL INGESTION OF LOW DOSE ASPIRIN (ASA).** J.C. Veille, R. Hanson, M. Sivakoff*, M. Swain, L. Henderson, Dept. of Ob/Gyn, Bowman Gray School of Medicine, Winston-Salem, NC and CWRU, Cleveland, OH*.

Chronic maternal ingestion of low dose ASA may have significant effects on fetal intracardial blood flow velocity (IBFV). Even though it has been assumed that no or minimal effects occur in the fetus, data are lacking. IBFV using pulsed Doppler was assessed at 3 different gestational age (GA) periods: Group (GRP) I = 12-23; II = 24-32; III = 33-41 weeks) in fetuses exposed to ASA. Fetuses not exposed to ASA were used for control (C). A total of 230 studies (gge; 51 ASA) were done. Results are as X ± SEM. An ANOVA was used to assess any differences between each GRP.

GRP	$TVTVI_C$	$TVTVI_{ASA}$	$MVTVI_C$	$MVTVI_{ASA}$
I	4.4 ± 0.2	3.9 ± 0.3	4.9 ± 1.2	3.7 ± 0.2*
II	6.1 ± 0.2	5.9 ± 0.3	6.5 ± 0.9	5.5 ± 0.4*
III	7.1 ± 0.2	8.1 ± 0.5	6.1 ± 0.2	5.9 ± 0.5
p	< 0.000	< 0.0000	NS	.002

GRP	$TVPFV_C$	$TVPFV_{ASA}$	$MVPFV_C$	$MVPFV_{ASA}$
I	41.5 ± 2	37.4 ± 2	36.9 ± 1*	33.9 ± 1*
II	50.7 ± 2	45.7 ± 2	46.9 ± 12*	42.6 ± 8*
III	56.6 ± 1	60.3 ± 3	46.7 ± 1	43.5 ± 4
p	< 0.0000	< 0.000	0.00	.0000

Legend: TVTVI = Tricuspid valve time velocity integral; MV = Mitral valve; PFV = Peak flow velocity (cm/sec); C = control; ASA = Aspirin. Results: (1) PFV and TVI across the tricuspid valve ↑ significantly with advanced GA in both the C and ASA. No difference within the groups was found. (2) MV TVI did not significantly ↑ with GA in the C GRP. In the ASA, TVI significantly ↑ during the 2nd GA period. No difference was documented, however, within each GA group. (3) MVPFV significantly ↑ in the C and ASA GRPS during the 1st to 2nd GA period. Conclusions: Even though major changes were seen in intracardial blood flow with advancing GA, NO DIFFERENCE was documented between the C and ASA GRPS. (Supported by NIH Grant HL38296).

202 **LOW MIDDLE CEREBRAL ARTERY (MCA) RESISTANCE INDEX OF POURCELOT (RI) PREDICTS NEONATAL MORBIDITY IN POST-TERM PREGNANCIES.** J. Shyken, S. Lieberman[x], A. Kivikoski[x], J. Smeltzer, Dept. OB/GYN, Washington Univ., St. Louis, MO

Uterine and umbilical artery velocimetry has not proven useful in management of prolonged pregnancies. There is little information regarding the value of MCA Doppler for this purpose. Forty-seven well-dated pregnancies at ≥41 weeks gestational age were prospectively studied to define the relationship between fetal cerebrovascular resistance as measured by MCA-RI, umbilical artery pH, and occurrence of abnormal pregnancy outcomes. Two groups were identified: those with normal outcomes and those with neonatal morbidity (having two of the following: umbilical artery pH <7.2, low 1 minute or 5 minute Apgar score, or neonatal intensive care unit admission). Eight of 47 infants had significant neonatal morbidity. The normals differed from the abnormals only by mean MCA values (0.74 ± .01 versus 0.64 ± .03, t=2.98, p<.005) and gestational age in weeks (41.8 ± .08 versus 42.3 ± 0.1, t=-2.58, p<.02). Gestational age and MCA-RI together clearly delineated those with neonatal-morbidity from others by discriminant analysis. Cutoff values for MCA-RI and their corresponding systolic to diastolic ratios (S/D) for each gestational age were calculated (using the formula RI = 0.14(gestational age) - 5.16) to obtain 100% sensitivity.

Weeks Gestation	RI	S/D
41	≤.58	2.38
42	≤.72	3.57
43	≤.86	7.14

For these cutoff values, sensitivity was 100%, specificity 74.4%, and positive predictive value of 44.4%. Further, MCA-RI predicted umbilical artery pH (R^2=0.22, p=.03). CONCLUSION: MCA Doppler indices are helpful in the management of post-dates pregnancies, and low RI values as indicated portend neonatal morbidity.

203 **UTERINE ARTERY DOPPLER VELOCIMETRY, PLACENTAL PATHOLOGY AND PERINATAL OUTCOME.** E. Ferrazzi [x], G.P. Bulfamante [x], A. Barbera [x], A. Pavesi [x], and G. Pardi. Dept. Ob/Gyn, Dept. Pathology, San Paolo Biomedical Science Institute, University of Milan, Italy.

The placental morphology was analyzed in 21 pregnancies with abnormal longitudinal uterine doppler S/D ratio (abn. S/D) and in 15 pregnancies with normal uterine S/D ratio (nor. S/D). Nine patients with abn. S/D developed pregnancy induced hypertension (PIH). All fetuses with abn. S/D were growth retarded in utero (IUGR). 3 fetuses only with nor. S/D were IUGR (see Table-top). In the normal pregnancy-nor. S/D group, only 8% of the placentas showed haematoma and 17% had trophoblastic hischemic lesions. IUGR fetuses with nor. S/D showed similar normal placentas. In the IUGR group with abn. S/D, placentas showed similar lesions to those observed in IUGR-PIH group with abn. S/D.: large infarcts (41% vs.55%), abruptio placenta (8% vs 22%), placental hematoma (25% vs 22%) and trophoblastic hischemic changes (83% vs 100%). Table-body shows the perinatal results in the 4 groups:

PREGNANCY	NORMAL	IUGR	IUGR	IUGR - PIH
UTERINE S/D	NOR.	NOR.	ABN.	ABN.
N. CASES	12	3	12	9
ABN.UMBILICAL PI	-	-	7	7
FETAL DISTRESS	-	-	5	3
P.N. DEATH	-	-	1	2
DELIVERY WEEK	38(2)	39(1)	33(3)	29(2)
CESAREAN SECT.	-	-	12	7
NEWBORN WEIGHT	3195(456)	2323(230)	1581(545)	896(376).

IUGR with abn. S/D are at greater risk of placental lesions and related abnormal outcome.

204 **THE PERINATAL SIGNIFICANCE OF ABSENT END DIASTOLIC FLOW IN DISCORDANT TWINS.** E. Baker,[X] J. Crowley,[X] K. H-Wilkes,[X] M. D'Alton, Dept. of MFM, St. Margaret's Hospital, Tufts University, Boston, MA

Over two years 50 pairs of twins demonstrated discordant fetal growth by ultrasound with intrapair estimated fetal weight difference of at least 20%. All sets had serial growth scans and umbilical artery Doppler studies. Of the 50 pairs 37 had normal Dopplers. 13 pairs demonstrated absent end diastolic flow (AEDF) in the smaller twin (4 with dichorionic and 9 with monochorionic placentation.) AEDF did not dictate delivery; however, intensive fetal surveillance with daily NST's AND BPP's were suggested by the perinatal team. The average gestational age at delivery was comparable between the groups with and without AEDF (33.0 wks. vs 33.8 wks.) There was no significant difference between discordancy at delivery in the group with AEDF (26% ± 13%) and the groups with normal Doppler studies (25% ± 9%.) The overall perinatal survival was 94/100 (94%.) There were 6 perinatal deaths (4 IUFD's and 2 neonatal deaths) in the twins with AEDF compared with no deaths in the group of discordant twins with normal Doppler flow (p<0.001.) An additional 2 cases demonstrated multicystic encephalomalacia and seizures in the neonatal period. No serious neonatal morbidity occurred in the group with normal Doppler flow. In conclusion AEDF identifies a subset of discordant twins who are at significant risk for perinatal death and adverse neonatal outcome.

205 **COLOR AND SPECTRAL DOPPLER FLOW MAPPING OF THE HUMAN PLACENTA** *S Rotmensch, M Liberati,* JS Luo,* Y Gollin,* JC Hobbins. Dept OB/GYN, Yale University*

Color Doppler flow equipment allows for visualization of arterial, venous and capillary flow in trophoblast and placenta' bed. We investigated morphologic, and blood flow characteristics of subplacental spiral arteries and intravillous fetal arteries in subsets of 74 normal (NL) and 13 IUGR pregnancies between 18 and 41 weeks gestation. Fetal vessel counts were standardized on a 2 cm^2 ultrasound screen area (SA). *In vivo* and histopathologic findings of placental tissue and placental bed biopsies were correlated in 2 cases. **Results:** 1.Mean intravillous vessel count was not different between normal and IUGR placentas. However, in one severely distressed IUGR fetus no intravillous flow could be detected with maximal gain settings. 2.Pulsatility index (PI) decreases in fetal circulation from umbilical artery (UA) towards central placenta (CP) ($p<0.001$) and apparently continues to decrease towards peripheral placenta (PP). Measurements in scanning planes parallel to blood flow suggest that this gradient is due to relatively increased diastolic flow in the PP. PI of intravillous arteries appeared to be higher in IUGR placentas ($p=0.06$, $n=13$). However, forward diastolic flow was observed in intravillous arteries even when flow in the UA was absent or reversed. 3. In one case of severe IUGR, the usually prominent decidual vascular turbulence was remarkably diminished, and correlated with absent "physiologic change" on placental bed biopsy. Mean PI of subplacental spiral arteries was 0.46 ± 0.14 between 20-30 wks gestation. 4. Pulsatile maternal blood flow into 2/3 of placental mass thickness was observed, contrary to the Ramsey theory. **Conclusions:** Color Doppler flow mapping of the placenta allows for *in vivo* investigation of hemodynamic aspects and might be a useful adjunct in the assessment of utero-placental function.

206 UMBILICAL ARTERY VELOCITY DECELERATION TIME IN FETUSES WITH ABSENT END-DIASTOLIC VELOCITIES. JH Indik,[X] KL Reed, Arizona Health Sciences Center, Tucson, AZ

Fetuses with absent end-diastolic velocities in the umbilical artery (AEDV) and umbilical venous pulsations have a worse outcome than fetuses with AEDV without umbilical venous pulsations. To quantify the association between umbilical arterial and venous velocities, we examined umbilical artery velocity deceleration time (time from peak velocity to time of estimated zero-line intercept of the deceleration slope) and divided by the time of the cardiac cycle in 21 fetuses with AEDV. Percent deceleration time in 11 fetuses with umbilical venous pulsations was decreased (0.47±0.12%) compared with 10 fetuses without umbilical venous pulsations (0.58±0.08%, $p<0.05$). The decreased percent deceleration time in the fetal umbilical artery may be due to increased afterload in the placental and fetal venous vasculature, with a mismatch between volume flow and vascular compliance. These results are further evidence of the interrelationship of umbilical arterial and umbilical venous velocities, since umbilical venous pulsations are associated with changes in umbilical arterial velocities. In addition, a subgroup with decreased percent deceleration time and increased morbidity is identifiable in fetuses with absent end-diastolic velocities in the umbilical artery.

207 USE OF DOPPLER IN THE MANAGEMENT OF HYPERTENSIVE DISORDERS IN PREGNANCY

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Although there are many studies on the management of hypertension during pregnancy,there are no standards which analyze how management effects decision making for: usage of drugs, tests, allowing or inducing labor,and the frequency of cesarean section. In this study we present a 2 year experience from a community teaching hospital in which Doppler velocimetry formed the basis for the management of pregnant women with hypertension. From 1/1/89 through 12/31/90, 134 hypertensive patients were evaluated with uterine and umbilical Doppler velocimetry, and results are as follows:

Doppler	Both Normal	Abnormal Uterine	Abnormal Umbilical	Both Abnormal
Maternal Fetal Parameters				
#Of Pts	104	13	12	5
Mean Age(yrs)	30±5	30±6	28±6	34±4*
Nullipara(%)	37	23	67*	20
GA Del(wks)	39±2	38±3	36±2***	32±3***
Bthwt(gms)	3255±657	2818±970	2085±827***	1353±42***
IUGR(%)	8	15	33	40
NICU(%)	12.5	31	58**	100***
Antepartum Management				
Bedrest(%)	32	62*	66*	100***
BPmeds(%)	23	15	42	80**
SeizureMeds(%)	12	15	50*	40
AP LOS(days)	13±11	7±3***	13±12	9±10
Labor/Delivery Management				
Labor(%)	90	77	83	0***
Spont(%)	72	70	40*	0***
Induct(%)	28	30	60*	0***
C/S(%)	34	62*	58	100***
CS NoLabor	29	38	29	100***
CS for HTN	10	33	100***	100***

*p<0.05 **p<0.01 ***p<0.001

A Doppler classification of hypertension in pregnancy identifies 4 groups of patients with varying clinical outcomes. The classification distinguishes women who can be managed expectantly from those who have increasing fetal risk. We offer this study as a beginning effort for others to analyze the effect of various management plans on the need for prenatal testing, medications hospitalization, inductions of labor and cesarean sections.

208 MIDDLE CEREBRAL ARTERY BLOOD FLOW VELOCITY IN PREGNANCIES WITH EXTREMELY ABNORMAL UMBILICAL ARTERY S/D RATIO

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Management, specifically the timing of delivery, of patients with extremely abnormal umbilical artery S/D ratio remains controversial. Six patients with extremely abnormal umbilical artery S/D ratio (defined as S/D > 99th percentile and/or absent end diastolic velocity) were diagnosed during a 3 years period. All patients had risk factors requiring conventional antenatal surveillance. Middle cerebral artery (MCA) flow velocimetry was performed regularly starting from the time of diagnosis of abnormal umbilical artery flow (24-28 weeks of gestation) until the time of delivery. The physicians managing the pregnancy were blinded to the MCA flow velocity measurements. Upon first measurement, all MCA flow velocities were abnormal (<mean - 2SD). All patients delivered small for gestational age infants with a neonatal mortality rate of 33%. All six patients delivered prior to 35 weeks of gestation because of deteriorating antepartum fetal testing. Analysis of MCA flow velocity revealed that all of the waveforms converted to normal on an average of 4 days prior to worsening of conventional antepartum fetal testing necessitating delivery. The umbilical artery S/D ratios remained extremely abnormal despite normalization of MCA flows. **Conclusions:** In patients with abnormal umbilical S/D ratios, abormal MCA flow velocities may predict eventual poor outcome earlier than conventional testing. Abnormal MCA flow velocity, however, did not indicate a need for immediate delivery. In fact, a return of MCA flow velocity to normal was a harbinger of impending fetal jeopardy. The role of MCA flow velocity measurement in optimally timing the delivery of the at risk fetus requires further investigation.

209 CLINICAL SIGNIFICANCE OF MEASURING THE UMBILICAL ARTERY S/D RATIO AT THE PLACENTAL AND FETAL CORD INSERTION SITES

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Umbilical artery S/D ratios vary at different sites along the umbilical artery. We studied a group of fifty high risk pregnancies in the third trimester. We analyzed the ability of the umbilical artery S/D ratio, performed within 7 days of delivery, to predict the likelihood of poor pregnancy outcome among these patients. Poor outcome was defined as small -for- gestational age infants, the presence of meconium at delivery, fetal distress in labor requiring cesarean section, or 5 minute Apgar scores < 7. Twelve patients, (24%) had abnormal S/D ratios near the abdominal end of the umbilical cord. Abnormal S/D ratios were seen in 9 (18%), and 4 (8%) patients at the mid cord segments and placental insertion sites, respectively. All those with an abnormal S/D ratio at placental end of the cord also had an abnormal S/D ratio at the mid cord segment and abdominal insertion site. This, however, was not true for patients who had abnormal measurements at the mid cord or abdominal end of the cord. Poor outcome occurred in 11 patients (22%). An abnormal S/D ratio (> mean + 2SD) at the placental insertion site had a better predictive value (40%) than did abnormal values at the mid cord and abdominal end of the cord (28% and 20% respectively). **Conclusion:** In our study, the ability to use Doppler blood flow studies to predict poor pregnancy outcome is less than reported in the obstetric literature. Our success in predicting adverse outcomes, however, was improved by measuring the umbilical artery S/D ratio at the placental insertion site.

210 TRICUSPID REGURGITATION: A METHOD OF MONITORING PATIENTS TREATED WITH INDOMETHACIN.

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Doppler ultrasound (U/S) has been used to detect fetal ductus arteriosus constriction in up to 50% of patients treated with Indomethacin. This technique is difficult to perform and results are not entirely reproducible. In this study, we propose an alternate method of monitoring patients on Indocin. Significant ductal constriction will lead to tricuspid valve regurgitation (TR). Using Doppler U/S to detect TR, 30 normal control patients were studied to determine the typical velocity waveform that occurs across the tricuspid valve. The upper limit of normal for regurgitant flow was determined to be .6 m/sec. 45 patients in the same gestational age range who were treated with Indocin (50 mg suppository load, 25 mg PO q 6) for preterm labor were studied using the same technique. Length of therapy was variable, but all patients had at least one study performed per 72 hour interval. Indocin was discontinued if there was significant regurgitant flow or the amniotic fluid index became ≤ 5 cm. Results: There were 2 cases (4.4%) of TR detected and 4 cases (8.8%) of oligohydramnios (oligo). Both cases of TR resolved within 24 hours and 3 of 4 cases of oligo resolved with 72 hours. The fourth case with oligo delivered within 24 hours of stopping Indocin. There were 31 neonatal intensive care unit admissions. There were no instances of persistent fetal circulation or intraventricular hemorrhage. One infant developed mild necrotizing enterocolitis at 30 days of life and one infant developed transient renal tubular dysfunction. The etiology of the renal failure was thought to be related to either gentamycin or Indocin toxicity. Neither of these cases was from the group that developed TR or oligo. We conclude that patients treated with Indomethacin can be safely and easily monitored by observing for the development of TR or oligo and that the incidence of clinically significant ductal constriction may be less than originally reported.

211 **COMPARISON OF THE DOPPLER WAVEFORM CHARACTERISTICS OF THE PROXIMAL VERSUS DISTAL UTERINE ARTERY USING COLOR FLOW MAPPING.**

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Analyses of Doppler ultrasound flow velocity waveforms (FVWs) have been used to investigate the uteroplacental circulation. Previous studies suggest that uterine artery FVWs vary significantly with the sampling site. **Purpose:** To determine whether the location along the uterine artery alters the doppler FVWs and to assess whether observed differences are influenced by gestational age (GA). **Methods:** 27 subjects from 18 to 40 weeks GA had studies performed. 15 subjects had repeated studies at 4 week intervals. The proximal and distal UA were identified with color flow mapping. Pulsed Doppler was then used to obtain the FVWs. The mean S/D ratio and RI were calculated from at least 3 waveforms. The data were analyzed using repeated measures analysis of covariance with and without GA as the covariate and the subject as a random factor for repeated measures.

Results: n= number of studies

ANCOVA* For RI and S/D Proximal VS. Distal

	n	mean diff. (SEM)	p-value
S/D placental	50	0.30 (0.06)	.0001
S/D non-placental	51	0.50 (0.21)	.021
RI placental	50	0.09 (0.02)	.0001
RI non-placental	51	0.07 (0.02)	.0001

*Analysis of covariance without gestational age in the model.

Gestational age was not an important covariate in any of the analyses. The changes between proximal and distal RI and S/D ratios were not significantly different between placental and non-placental sides. **Conclusions:** The S/D ratio and RI decline significantly along the course of the UA and this decline is not influenced by GA. This decline may reflect a decrease in resistance to flow as the uterine vessels near the placenta. Supported by UCTRDRP Grant#1KT96

212 EFFECT OF SAMPLING SITE ON UMBILICAL DOPPLER INDICES IN INTRAUTERINE GROWTH RETARDATION (IUGR)

A. Skoll, S. Sonesson[X], G. Tessyler[X], P. Bonnin,[X] J.-C. Fouron[X]. Fetal Cardiology Unit, University of Montreal, Montreal, Quebec.

Previously, we confirmed that the S/D ratio, pulsatility index (PI), and resistance index (RI) are higher in the abdominal than placental end of the umbilical artery in normal pregnancy, and that this difference disappears at the end of gestation (International Perinatal Doppler Society, 1991). We hypothesize that this is due to a relative increase in fetal circulatory volume compared to placental volume late in gestation. Therefore, we sought to determine whether this pattern holds true for growth retarded fetuses. Systolic and diastolic velocities were measured at the abdominal (A) and placental (P) ends of the cord of 28 fetuses 28 - 40 weeks gestation with severe IUGR (birthweight < 3rd percentile for GA). The mean A-P difference in all indices remained significant even at the end of gestation. (Table 1) This suggests: 1) that when monitoring these high risk fetuses with doppler studies, sample site should be controlled for in serial examinations, and 2) that the disappearance of the A-P difference in normal fetuses may result from increased fetal blood volume relative to placental volume late in gestation.

Table 1 Mean A-P difference in indices (>36 wks)

	Δ S/D	Δ PI	ΔRI
Normals	0.29±.6†	0.05±.17†	0.03±.07†
IUGR	1.17±.99*	0.33±0.2*	0.10±.08*

† = NS

* = p<.05

213 LOW PLACENTAL RESISTANCE IS ASSOCIATED WITH FETAL MACROSOMIA. AL Diket[X], HA Gabert and JM Miller, Jr., LSU Medical Center New Orleans, LA

We evaluated the relationship of umbilical artery Doppler velocimetry and increased fetal growth. 153 patients who delivered ≥38 weeks were studied with continuous wave Doppler within 2 weeks of delivery. Large for gestational age (LGA) fetuses were compared to appropriate for gestational age fetuses (AGA). Repetitive measurements of the systolic to diastolic ratio (S/D), pulsatility index (PI) and resistance index (RI) were averaged. Gestational age at time of study and delivery was not different between the two groups. S/D, PI and RI were significantly less in the LGA group.

	Weight(g)	S/D	PI	RI
LGA	4212±212	2.20±.25	.82±.14	.54±.05
AGA	3207±356	2.46±.37	.95±.20	.58±.05
p	.0001	.0001	.0001	.0001

Individual evaluation of each of the 3 measurements found that sensitivity and specificity were poor.

S/D	LGA	AGA	PI	LGA	AGA	RI	LGA	AGA
≤2.00	9	5	≤.75	11	10	≤.50	10	7
>2.00	30	109	>.75	28	104	>.50	29	107

While low values are poorly predictive of LGA status, the decreased resistance observed may identify one mechanism of macrosomia.

214 AMNIOTIC FLUID GLUCOSE CONCENTRATIONS ARE REDUCED IN TERM PARTURITION AND IN CYTOLOGIC AMNIONITIS. R. Romero, M. Famirez,[X] W. Sepulveda,[X] F. Brandt,[X] R. Gonzalez,[X] E. Behnke,[X] M. Mazor, Depts. of Ob/Gyn; Yale Univ. School of Medicine, New Haven, CT; Wayne State Univ., Detroit, MI

Amniotic fluid glucose (AF-G) determinations have been proposed as a sensitive method for the diagnosis of intraamniotic infection (AJOG 1990;163:968 and AJOG 1991;164:818). Recent observations, however, indicate that labor per se may decrease AF-G concentrations and thus limit the utility of this test (SGI 1991, Abs. #84). This study was designed to examine the effect of term labor and cytologic amnionitis on AF-G levels. Materials and Methods: AF was retrieved from 365 women divided into four groups: group 1, term patients not in labor (n = 101); group 2, term patients in active labor with an AF white blood cell count (AF-WBC) of <50 cells/mm^3 (n = 184); group 3, term patients in active labor with an AF-WBC of >50 cells/mm^3 (n = 30); group 4, women in the midtrimester of pregnancy undergoing genetic amniocentesis (n = 50). Glucose determinations were performed by the glucose-oxicase method. Patients at term had simultaneous plasma glucose determinations. Results: 1) Women in active labor had significantly lower AF-G than women at term not in labor (median = 8.9 mg/dL, range = 3.8-38.8 vs. median 13.5 mg/dL, range 4.8-33.8; p <0.0001). 2) Women in active labor with an AF-WBC >50 cells/mm^3 had a significantly lower AF-G than those with an AF-WBC <50 cells/mm^3 (median = 9.4 mg/dL, range = 3.8-38.8; p <0.0001). 3) There were no significant differences in maternal plasma glucose concentrations among the three study groups (p = 0.57). 4) Patients in the midtrimester of pregnancy had a significantly higher AF-G concentration than patients at term (median = 43.1 mg/dL, range = 26.5-62.8 vs. median = 13.5 mg/dL, range = 4.8-33.8; p <0.05). Conclusions: 1) Normal AF-G concentrations decrease with gestational age. 2) The cut-off of 14 mg/dL proposed for the diagnosis of intraamniotic infection in preterm labor cannot be used at term because at least 50% of normal women had an AF-G concentration below this level. 3) Term labor is associated with a reduction in AF-G concentration. 4) Patients with cytologic amnionitis had the lowest AF-G concentration observed in this study.

215 EXTRACORPOREAL PERFUSION OF HUMAN UTERI: A SYSTEM TO STUDY THE ELECTROMECHANICAL EVENTS OF MYOMETRIAL CONTRACTILITY. C. Bulletl,[X] G. Bazzochl,[X] R. Romero, R.A. Prefetto,[X] P. Mimmi,[X] G.A. Lanfranchi,[X] C. Flamigni, Depts. of Ob/Gyn, Yale University School of Medicine, New Haven, CT and The University of Bologna, Bologna, Italy

Studies of the physiology and pharmacology of human uterine contractility are essential for understanding the mechanisms involved in term and preterm labor. Previous experimental approaches to the study of myometrial contractility have been based upon either myometrial strips or animal models. Studies based upon myometrial strips are non-physiologic because the myometrium is removed from paracrine control mechanisms. Extrapolation of results of animal studies to the human is always open to question. We have designed a new experimental system that uses extracorporeal perfusion of human uteri to study the electromechanical events of uterine contractility. Materials and Methods: Uteri were obtained (n = 10) after total abdominal hysterectomy. Uterine arteries were cannulated and connected to an extracorporeal perfusion machine specially designed for uterine perfusion. This system used a servomechanism to maintain perfusion pressure within physiological range (120 mm Hg +/- 10 mm Hg), temperature (37°C) and humidity (99%). The perfusion medium was Krebs-Ringer HCO3, 2% dextrose, pH = 7.4. Electrical activity was recorded by bipolar intramyometrial electrodes, and mechanical activity was recorded with an intraluminal fluid-filled open catheter connected to a Beckman type 4-237 transducer. To validate this system, we perfused the uterus with oxytocin and recorded electromechanical events. Results: Functional studies of human contractility could be carried out for at least 24 hours after establishment of the preparation. Oxytocin perfusion was associated with an increase in electrical activity (spike potentials/30 minutes, percent duration of rhythmic spike activity and frequency of rhythmic spike activity) (p <0.01) and also in the frequency of contractions per 10-minute window (p <0.01). Conclusion: A novel experimental paradigm for the study of human uterine contractility has been developed. This system can be used to examine the effect of uterotonins and tocolytic agents before administration to humans.

216 AMNIOTIC FLUID INTERLEUKIN-6 AND PROSTAGLANDIN E_2 ARE INCREASED IN TERM HUMAN PARTURITION. M. Mazor, R. Romero, D. Kleinman,[X] A. Wiznitzer,[X] M. Glezerman,[X] Departments of Obstetrics and Gynecology, Soroka Medical Center, Ben-Gurion University, Israel and Yale University School of Medicine, New Haven, CT

Cytokines have been implicated in the mechanisms responsible for preterm human parturition in the setting of infection. It is unclear, however, if cytokines may also play a role in term labor. Interleukin-6 (IL-6) is a cytokine produced by a variety of immune and nonimmune cells in response to several infectious and noninfectious stimuli. Recently IL-6 has been shown to stimulate prostaglandin (PG) production by human amnion and decidua (Eur J Pharmacol 1991;192:189). The purpose of this study was to examine whether there is a correlation between amniotic fluid (AF) concentrations of IL-6 and PGE_2. Materials and Methods: AF was collected from 19 women at term not in labor and 32 women in active spontaneous labor. IL-6 was measured with a radioimmunoassay (RIA) validated for AF and PGE_2 with an RIA previously described (J Pharmacol 1983;35:576). Results: Women in active labor had significantly higher AF concentrations of IL-6 than women not in labor (median = 2.58 ng/ml, range = 0.2-8.83 vs. median = 0.37 ng/ml, range = 0.1-2.05, respectively; p <0.001, Mann-Whitney U test). Similarly AF PGE_2 concentrations were significantly higher in women in active labor than in those not in labor (median = 6.46 ng/ml, range = 1.16-23.05 vs. median = 0.21 ng/ml, range = 0.07-0.68, respectively; p <0.001). A strong correlation between AF IL-6 and PGE2 was found (Spearman correlation, r = 0.89; p <0.001). Conclusion: Term human parturition is associated with a significant increase in AF of both IL-6 and PGE_2.

217 **A COMPARISON OF INTRAVAGINAL PROSTAGLANDIN E_2 AS A GEL OR CONTROLLED RELEASE PESSARY FOR CERVICAL RIPENING AND INITIATION OF LABOR.** C. Smith, A. Miller, M. Stancil, W. Rayburn. Dept of OB/GYN, Univ of Nebraska College of Medicine, Omaha, NE.

Intravaginal application of prostaglandin E_2 (PGE_2) in a low-dose preparation is helpful to promote cervical ripening and initiate labor. The purpose of this one-year investigation was to compare the safety and efficacy of PGE_2 administered either sequentially as a 2.5 mg gel (n=79) or as a single application of a controlled release 10 mg pessary (n=82). The groups were similar in maternal age, race, gestational age, initial Bishop score, and indication for induction. Cesarean section for a failed induction occurred in equal proportion between the two groups. A change to a Bishop score ≥ 8 occurred more often in the pessary than gel group at 6 hours (29, 35.4% versus 12, 15.2%, p = 0.003) and at 12 hours (55, 67.1% versus 38, 47.5%, p = 0.012) postdosing. Labor ensued without need for oxytocin more often in the pessary than gel group (68, 82.9% versus 40, 50.6%, P < .0005). Multiple dosings of the gel were required in 49 (62.1%) cases. Uterine hyperstimulation occurred with slightly greater frequency in those patients receiving the pessary than the gel (7, 8.5 % versus 1, 1.3% , P = 0.078), but this difference was not statistically different and removal was accomplished only with the pessary. We conclude that intravaginal PGE_2 is better delivered as a controlled release pessary than as a gel, because it is a singly applied, easily removable preparation which produces greater cervical change and often initiates labor without need for oxytocin.

218 **NEONATAL OUTCOME IN PROLONGED 2ND STAGE OF LABOR.** E. Xenakis,* O. Langer, N. Field,* A. Samueloff,* L. Ridgway, M. Berkus Dept. OB/GYN, Univ. Texas HSC at San Antonio, Texas.

It is commonly held that a 2nd stage of labor exceeding 2 hours is associated with increased morbidity. More recent data have challenged this concept. In order to investigate the relationship between length of 2nd stage and fetal outcome, 1670 consecutive women were studied. Adverse neonatal outcome was defined as: level III NICU admission with respiratory support and/or hypotonia, IVH, sepsis, convulsions or neonatal death. The study revealed that in 92% of the cases, fetal heart rate (FHR) characteristics could not predict adverse outcome. Only in 8% of cases was abnormal FHR associated with adverse outcome. The relationship between the length of 2nd stage (both nulliparas and multiparas) and abnormal outcome is shown below:

N	Duration of 2nd Stage (min)	Adverse Neonatal Outcome	Cord pH <7.20	Apgar at 5min <7
760	0-29	3.3%	10.3%	0.4%
224	30-59	1.3%	19.3%	0.4%
90	60-89	5.6%	26.7%	3.3%
37	90-119	10.8%	16.2%	---
45	120-179	4.0%	22.0%	-----
24	180+	20.8%	26.1%	16.7%

p < .0001

Further analysis (stepwise multiple regression) showed that 30% of the variance (prolonged 2nd stage) is explained by: length of 1st stage of labor; station at entry in 2nd stage; birth weight; and analgesia. Our data indicate a significant increase in the deleterious effects of prolonged 2nd stage on the fetus.

219 USE OF AN INSUFFLATABLE ABDOMINAL GIRDLE TO SHORTEN THE SECOND STAGE OF LABOR M.Y. Cai,* S.F. Zhao* (Sponsor: R. Petrie) Depts. OB/GYN. Peking Union Medical College, Beijing & Lishan Hospital, Liaoning, China

Prospectively 160 consecutive normal term nulliparas were randomized into study and control groups (80 each). During the second stage of labor, the abdominal girdle was insufflated to a basal pressure of 30-60 torr, during contractions the girdle pressure was increased and maintained at a general pressure of 150-200 torr, and with the appearance of 2-3 cm diameter of scalp at the introitus, the girdle was further insufflated to a high pressure of 300 -350 torr. Control patients were managed identically except without girdle. The mean ages were 26.2 (control) and 26.6 (study). The mean durations of second stage labor were 40'23"(S) and 59'59"(C) P<0.01. The mean durations from appearance of fetal head at the vulva to complete delivery were 11'56"(S) and 20'56"(C) P<0.01. Vacuum extraction was necessary in 15/80 instances(C) and 5/80(S). The mean 1 minute Apgar scores were 9.98(S) and 9.88(S) P>0.05. Funic (A&V) respiratory blood gas values were within normal ranges as were maternal vital signs and respiratory blood gas analyses P>0.05. There were no differences in the instances of abnormal FHR patterns or presence of meconium between the two groups. The results of this study indicate that this technique is simple, safe and easy to use as well as an effective procedure for both shortening second stage labor and reducing the need for vaginal operative delivery safely for mother and newborn.

220 **INTRAAMNIOTIC 15 METHYL PROSTAGLANDIN F2 ALPHA VERSUS INTRAVAGINAL PROSTAGLANDIN E2 FOR SECOND TRIMESTER LABOR INDUCTION**

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Second trimester abortion is frequently complicated by significant morbidity. Although instrumental dilatation and evacuation is touted to be the safest method of second trimester abortion, many physicians lack the experience to perform this procedure confidently. This has led to the common use of prostaglandins as abortifacients. Unfortunately, frequent systemic side effects and a high incidence of incomplete abortion requiring surgical intravention are common complications. In an attempt to minimize these risks, we compared patients undergoing labor induction with vaginal prostaglandin E2 (Group A, N = 20) and intraamniotic 15 Methyl prostaglandin F2 alpha (Group B, N = 20). Group A received vaginal 20 mg PGE_2 suppositories every 3 to 4 hours. Group B received 2.5 mg 15MPGF_2 alpha intraamniotically under ultrasound guidance. Group A successfully delivered by 24 hours in 90% and by 36 hours in 100% of cases. Seven patients in Group A had an incomplete abortion requiring surgical intervention. Group B had 95% and 100% of patients delivered within 24 and 36 hours respectively and only 1 patient had an incomplete abortion. Significant gastrointestinal side effects were noted in 65% of Group A and 5% of Group B. (P < 0.001).

Table	Group A (N = 20)	Group B (N = 20)	P
Nullip/Multip	11/9	11/9	NS
Gest. Age	19.0±2.6 wks	18.0±2.5 wks	NS
Time to delivery	16.0±5.1 hrs	12.7±6.2 hrs	NS
Delivery of placenta	69.3±47.8 min	15.9±31.9 min	<.001
Side effects: GI	13 (65%)	1 (5%)	<.001
Fever.	6 (30%)	0	<.01
Incomplete Ab	7 (35%)	1 (5%)	<.01
Est. blood loss	230 cc	120 cc	NS

This study confirms the efficacy of intraamniotic 15 Methyl PGF_2 alpha for second trimester labor induction compared to intravaginal PGE_2 suppositories with significantly fewer systemic side effects and fewer cases requiring surgical intervention.

221 **Withdrawn at authors' request.**

222 EFFECT OF TIME INTERVAL BETWEEN TWIN DELIVERY ON OUTCOME. T. Feng,* R. Swindle,* Dept. Gyn/Ob, Johns Hopkins University School of Medicine, Baltimore, MD 21205

It has been recommended that the interval between delivery of twins should preferably be within 15 minutes (min) and not more than 30 min. The purpose of this study is to evaluate the effect of delivery interval on the outcome of the 2nd twin. All twin deliveries from 1981 to 1987 were reviewed for the following criteria: 1) infants weighed >1500 gram or were ≥34 weeks by Dubowitz score, 2) the first twin was delivered vaginally. Obstetric data obtained by chart review included mode of delivery, presentation of 2nd twin, interval between delivery and 5 min Apgar score. In addition, all infants with a 5 min Apgar score ≤7 had a chart review. Time intervals were divided into 4 categories ≤15 min, 16-30 min, 31-60 min and >60 min. These groups were later collapsed during analysis. The results were analyzed using Fisher's exact test to compare groups and a p-value <0.05 was considered significant. **Conclusion:** An interval >60 min between twin deliveries did not have an adverse effect on outcome as judged by Apgar score, length of stay, or birth trauma. The largest incidence of cesarean section (C/S) (50%) occurred in the 31-60 min time interval which may represent adherence to older recommendations regarding the "safe" interval between twin deliveries. Additional data will be presented.

	INTERVAL IN MINUTES				P VALUE	
EVENT	≤15	16-30	31-60	>60	≤30 vs >30	≤60vs>60
Total	59	33	16	11	--	--
Breech del. of 2nd twin	26	3	0	1	.0075	.1793*
C/S for 2nd twin	1	7	8	2	.0009	.5212*
2nd twin with Apgar <7	2	1	0	1	.6481*	.3251*

*Not significant by Fishers Exact Test

223 SERUM α_1- ANTI-TRYPSIN: A MARKER FOR LABOR.

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The association between labor status and an acute phase protein, α_1-anti-trypsin, also called α_1-anti-protease (API) was examined. Sera were obtained from 229 women on admission to the labor floor. Labor status was determined by Friedman curves. Concentrations of API were assayed by radial immunodiffusion using a monospecific antibody.

Results:

α_1-anti-protease in mg/dl

	membranes intact	ROM	combined
no labor	(N= 49) 236±6*	(N=11) 257±16	(N=60) 243±6*
latent phase	(N= 66) 255±7	(N=47) 252±9	(N=113) 258±5
active labor	(N=33) 259±11	(N=23) 269 ± 10**	(N=56) 259±7

* p = <.01 ** p = <.005

Gestational age, hypertension and diabetes had no obvious impact on our findings. The exact mechanism causing API elevation is speculative. However, we suggest that API may prove useful as a biochemical marker for labor, especially when membranes are intact.

224 **IS PRETERM LABOR AN INFLAMMATORY PROCESS? AMNIOTIC FLUID cICAM-1 LEVELS ARE ELEVATED IN PRETERM LABOR.** CM Salafia[x],CA Vogel[x], J Pezzullo[x], M Lentner[x], E Mainolfi [x], JP Burns[x], G Foye, P Swift [x], LSilberman[x] R Rothlein[x] Depts. Lab. Med. and Ob/Gyn, Danbury Hospital, CT, Boehringer Ingelheim Corp,CT,TRC, Rhode Island Hospital, RI.

Acute intrauterine infection and histologic markers of chronic inflammation of placental villi or implantation site have been linked to preterm birth. Acute infection is thought to initiate labor via cytokine induction of prostanoids. Cytokines in amniotic fluid (AF) in cases of preterm birth without histologic acute infection have not been detected. cICAM-1 is a molecule produced in the early stages of inflammatory responses, serum levels of which are increased in liver and cardiac transplant patients undergoing allograft rejection. We assayed cICAM-1 levels [cICAM-1] by ELISA in 40 AF samples obtained at amniocentesis at 16-17 weeks for maternal age/anxiety, and compared them to 20 AF samples obtained by amniocentesis for evaluation of maturity or at cesarean section (C/S) with delivery at 32 -36 weeks, and 18 AF samples obtained at term C/S delivery. Log-transformed data are presented as mean +/- SE. Mean [cICAM-1] in **midtrimester** samples was **36.8 pg/ml +/- 8.** Mean [cICAM-1] in **preterm births** was **419.5 pg/ml +/- 65.7 (T=5.28, p<0.05)**, and significantly elevated over that of **term samples** (mean [cICAM-1]=**173.3 pg/ml +/- 32.7 , T= 3.16, p<0.05).** 11 of 20 patients delivering preterm presented in spontaneous labor; mean [cICAM-1] was 598.8 pg/ml +/- 37 v. 347.2 pg/ml +/- 67 in 9 patients delivering without labor by C/S (p<0.05). AF [cICAM-1] in preterm and term cases was not associated with presence or severity of histologic acute intrauterine infection (p>.10). These data suggest that preterm labor is an inflammatory process and that chronic inflammation may contribute to otherwise idiopathic preterm labor. Also, disease processes which necessitate premature delivery of the fetus may also involve chronic intrauterine inflammation.

225 **PRESSURE WAVE CHARACTERISTICS OF THE UPPER AND LOWER UTERINE SEGMENT IN ARRESTED LABOR.** F.Margono,* A.Karimi,* E.Prakash* and H.Minkoff.Dept.Ob/Gyn,State University of New York, Brooklyn,NY

Most methods for the measurement of uterine contractions during labor involve the use of an intrauterine pressure transducer (IPT). Using a single IPT Seitchik found no difference in the pressure wafe form characteristics of patients with successful or failed first stage of labor, either before or after oxytocin. Caldeyro Barcia stated that the intensity of the contraction diminishes from the top to bottom of the uterus in normal labor with the upper segment of the uterus contracting more strongly than the lower. It is possible that in cases of failed 1st stage of labor the lower segment of the uterus contracts more strongly than the upper. This phenomen would not be detectable with single IPT. To investigate this possibility two IPTs were inserted into the upper and lower segments of the uterus of laboring patients with cervical dilatation of at least 4 cm who were failing to progress. All patients had a single term vertex fetus, and EFW <4000 g. There were 3 primiparas and 5 multiparas. The location of the IPT tips was confirmed by ultrasound, both intrauterine pressure wave patterns were recorded simultaneously. Management of labor was based upon the pattern of the upper segment and labor progress. 3 patients underwent cesarean section for failure to progress. Prelimenary analysis of the intrauterine pressure wave forms (table 1) showed that those who underwent cesarean section had higher intrauterine pressures in the lower segment than the upper segment. The remaining patients showed greater pressures in the upper segment. Oxytocin increased intrauterine pressure in both upper and lower segments but did not change the gradient between segments. If these data are confirmed, pressure gradients may be used to gauge the likelihood of success of pitocin.

Table 1: Active Pressure Integral pre and post oxytocin

	(Pre) Upper	Lower	(Post) Upper	Lower
NSVD	2.79±0.33	2.17±0.45	3.19±0.49	2.47±0.45
CS	2.24±0.25	2.84±0.65	3.22±0.36	3.80±0.32

226 **PREGNANCY AFTER CESAREAN SECTION: RESULTS FROM A SINGLE CENTER.** Steven A. Friedman, MD,* Clare L. Cammarano, MD,* Russell K. Laros, MD. Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco (UCSF), San Francisco, California

We conducted a retrospective review of the UCSF Perinatal Database to determine whether an attempted trial of labor (TOL) after previous cesarean section is more desirable than an elective repeat cesarean section (ECS). Inclusion criteria were previous cesarean section, birth weight ≥750 g, and delivery at UCSF between 1 June 1976 and 31 December 1990. A total of 589 patients (including 8 with twin gestations) met these criteria. Three hundred six (52%) had an ECS and 283 (48%) had a TOL, of whom 177 (63%) delivered vaginally. The two groups were similar in age, parity, gestational age, and birth weight. Results are expressed as means±SD. Significance is defined as P<.05.

	TOL (SVD & CS)	ECS	P Value
Endometritis (%)	3.9	5.9	NS
Hospital stay (d)	3.5±2.2	5.5±3.1	<.0005
Δ Hematocrit (vol %)	-4.9±4.5	-3.2±3.3	<.0005
Transfusion (%)	2.5	1.6	NS
5-min Apgar ≤6 (%)	4.5	2.6	NS
Umbilical artery pH	7.25±0.08	7.26±0.07	NS
Neonatal intensive care (%)	7.7	8.1	NS

There were no maternal deaths nor uterine ruptures. Although statistically significant, the greater fall in hematocrit in the TOL group is clinically unimportant. These data support the American College of Obstetricians and Gynecologists' recommendation that a woman with a single previous cesarean section "be counseled and encouraged to attempt labor in her current pregnancy."

227 DILAPAN PLUS PROSTAGLANDIN E_2 VAGINAL GEL FOR CERVICAL RIPENING. Andrew Chao, David Plourd[X], Mai-Anh Doan[X]. Dept. Ob/Gyn, Santa Clara Valley Medical Center, San Jose, CA

We compared Dilapan dilators plus prostaglandin E_2 (PGE_2) gel to PGE_2 alone. Third-trimester induction patients with Bishop scores <5 were randomly assigned. Group 1 (n=25) received 1-6 dilators (median, 5) plus 3 mg PGE_2 gel. Group 2 (n=37) received gel alone. Dilators were removed in 4 hours. One or two more PGE_2 doses were applied at 4-hour intervals if the Bishop score was still <5; then oxytocin was begun. Parity, gestational age, and initial Bishop scores were comparable. Group 1 had a greater increment of Bishop score at 4 hours (median 3, range 0-11 vs. median 1, range 0-9; $p < 0.05$). However, the insertion-to-complete-dilatation intervals did not differ significantly (1267 min ± 638 SD vs. 1240 ± 549), nor did cesarean rates. Furthermore, the amnionitis/endometritis rate was higher in Group 1 (44% vs. 8%). This trial failed to justify the combined use of Dilapan and PGE_2.

228 **MORPHOMETRIC CORRELATES OF CEPHALOPELVIC DISPROPORTION.** R.N. Pollack[X], L. Goldstone[X], MY. Divon. Dept Ob/Gyn, The Albert Einstein College of Medicine, Bronx, NY.

The significant contribution of labor abnormalities to the increasing number of cesarean sections has focused interest on the diagnosis and management of these conditions. Morphometric factors associated with cephalopelvic disproportion (CPD) have reportedly included maternal height, weight and shoe size, as well as fetal macrosomia and macrocephaly. A simple antenatal predictor of CPD which integrates both maternal as well as fetal morphometric parameters has yet to be described. Purpose: To ascertain if the ratio of maternal height to fetal size is altered in pregnancies complicated by CPD. Method: 60 patients delivered by cesarean section (c/s) for active phase labor disorders and 138 matched controls who had spontaneous vaginal deliveries were compared. Morphometric parameters studied included maternal height, maternal weight, maternal body mass index (BMI) and symphyseal fundal height (SFH). A parameter which relates fetal size to maternal height was defined as the Maternal-Fetal Ratio [MFR = Maternal Ht (cms) + Symphyseal Fundal Ht (cms)]. Results: A significant correlation between the presence of an abnormal MFR (defined as MFR < 4.00) and delivery by cesarean section for active phase labor disorders was observed ($p < 0.000001$). The odds ratio (OR) of a patient with an abnormal MFR having a cesarean section for an active phase labor abnormality was 5.08 (95% CI = 2.6 - 10.0). As previously observed short maternal stature was also associated with an increased rate of c/s for active phase labor disorders. The OR of a patient < 160 cms. tall having a c/s for an active phase labor abnormalities was 2.21 (95% CI = 1.17 - 4.17). Maternal weight and BMI were not significantly correlated with delivery by c/s for labor abnormality. Conclusion: Of the various parameters studied, an abnormal MFR was most significantly correlated with delivery by c/s for active phase labor disorders. Prospective evaluation of the maternal fetal ratio in the prediction of CPD may be indicated.

229 **PROLONGED LABOR (>12 H) IS ASSOCIATED WITH A SIGNIFICANT DECREASE IN UMBILICAL ARTERY pH.** GM Morton[x], T C-S Foster[x], GJ Valenzuela. Dept Ob & Gyn SBCMC and Loma Linda Univ., California.

Prolonged labor (>12 h) has been described as having a detrimental effect on fetal outcome; however, in many of these studies the characteristics of the population have been poorly described. We report here on the influence of duration of labor on umbilical cord pH. 1110 women delivered at term were studied. All were without fetal or maternal complications, had normal electronic fetal monitoring strips, and delivered spontaneously. Cord blood gases were determined within 20 minutes of delivery. Fetal umbilical arterial and venous pH's were averaged on patients with durations of labor of <4, 4-8, 8-12, 12-16, and >16 hours. A progressive decline in arterial, but not venous, pH was noted; Apgar scores did not differ among the groups. The frequency of arterial pH<7.2 increased from 8.0% at 4 h to 18% at >16 h; arterial pH of <7.1 occurred in 2.6% of those delivering after 16 hours, which was also significantly different from the other four groups. We conclude that increasing duration of labor is associated with a fall in umbilical artery pH, although the number of infants with true acidosis is very low.

230 **PROLONGED SECOND STAGE AND PERINATAL OUTCOME: A CASE CONTROL STUDY.**

Luis Sanchez-Ramos, M.D., Patricia Schroeder, M.D.[X], Donna Briones, R.N.[X] University of Florida, Jacksonville, FL.

A prolonged second stage of labor i.e; greater than 2 hours, has been regarded as dangerous for the parturient and her infant. However, recent studies have challenged this conclusion. The purpose of this study was to evaluate the effects of a prolonged second stage of labor on perinatal and maternal outcome. From September 1990 to June 1991 during which time 4523 patients delivered at our institution, patients with prolonged second stage were diagnosed and identified by L&D personnel. For each study case, two controls were chosen for comparison. After discharge the charts (mothers and infants) were reviewed with particular emphasis on perinatal and maternal complications. A total of 271 patients were evaluated (71 study patients and 140 controls). Both groups were similar except for the duration of the second stage. The study patients had a significantly higher rate of cesarean delivery and at delivery the birthweight was greater. However, the incidence of operative vaginal deliveries was similar in both groups. No differences were noted in Apgar scores, cord gas values, or median length of NICU stay. Maternal complications were similar in both groups. We feel that the data does not support an arbitrary shortening of the second stage in the presence of a reassuring fetal heart rate and normal descent of the presenting part.

231 PRIOR CESAREAN BIRTH: RISK FACTORS ASSOCIATED WITH UTERINE RUPTURE.

Anna S Leung MD,* Richard M Farmer MD, Eleanor K Leung MD,* Richard H Paul MD. University of Southern California, Los Angeles, California

Trial of labor (TOL) after previous cesarean section (C/S) is actively advocated. The risk of uterine rupture is a major concern as one undertook TOL. Delayed diagnosis of uterine rupture could be catastrophic. A uterine rupture was defined as a uterine wall defect requiring emergency laparotomy or operative intervention for fetal distress or acute maternal bleeding with an estimated blood loss of greater than one liter. A case control study was conducted to possibly identify the risk factors associated with uterine rupture. The cases consisted of 33 patients who had undergone TOL with uterine rupture between 1987-1989. 33 women with prior C/S during the same period were randomly selected as controls.

	cases	controls	P value
age	27.4	28	N.S.
parity	1.9	2.1	N.S.
No. previous C/S	1.3	1.1	N.S.
No. vaginal birth after C/S	0.2	0.5	0.045
birth weight (grams)	3516	3554	N.S.
gestational age (weeks)	40.4	40.0	N.S.
No. with amnionitis	7	2	N.S.
total duration of labor (hours)	15.8	10.7	0.036
duration latent phase (hours)	10.6	6.6	N.S.
duration active phase (hours)	6.5	4.7	N.S.
duration second stage (hours)	2.0	1.3	N.S.
No. with protracted labor (PL)	18	4	0.000
No. on oxytocin	25	19	N.S.
No. with epidural anesthesia (EP)	15	13	N.S.
No. on oxytocin with PL	16	4	0.003
No. with EP and PL	10	3	N.S.
No. on oxytocin with EP and PL	9	3	N.S.

In conclusion, patients with uterine rupture had protracted and longer duration of labor. The use of oxytocin or epidural anesthesia alone did not seem to be associated with uterine rupture. However, patients on oxytocin with protracted labor had higher incidence of uterine rupture. Patients who were undergoing TOL with oxytocin usage should be definitively evaluated for protraction and arrest disorders.

232 **THE PHYSIOLOGY OF SQUATTING DURING LABOUR**

J Gardosi[x], Perinatal Research & Monitoring Unit, Queens Medical Centre, Nottingham, England

Squatting was the normal birthing posture in many cultures but on modern labour wards, women are rarely able to adopt this position. With the development of the Birth Cushion, squatting has become a viable option for the management of second stage and delivery. This obstetric device is made of a U-shaped soft foam wedge which the mother sinks into while pulling on side handles during the bearing down efforts. A previously reported randomised controlled trial of 427 women in their first labour showed that this posture, as compared to a semi-recumbent position, results in significantly shorter second stages (median 31 vs 45 min), fewer forceps deliveries (9 vs 16 %), fewer perineal tears but more labial tears, and no difference in blood loss. Further investigation into the physiological effects of this posture during second stage reveals that it affects all three obstetric 'P's of labour: *1. The Powers* are increased as bearing-down is easier, more efficient and better coordinated; this results in an increase in the voluntary component of intrauterine pressure during contractions. *2. The Passenger* is able to contribute more of his weight, as a continued downward force acting during as well as between contractions. *3. The Passages:* Previous, unconfirmed Xray studies have suggested that the pelvic outlet increases in upright postures. The effect of squatting on the bispinous diameter was investigated with vaginal ultrasound pelvimetry (n=13), using a Kretztechnik Combison 300 Panoramascanner with 230 degree vaginal probe. This showed a mean increase of 4.1 mm (semi-recumbent 107.2 mm, SD 4.6; squatting 111.3 mm, SD 7.0; paired t-test 3.989, p=0.002). Squatting has proven clinical as well as physiological advantages which help to explain the popularity that this birthing position has had throughout ancient history.

233 CHORIOAMNIONITIS: A HARBINGER OF DYSTOCIA. A.J. Satin,* M.C. Maberry,* K.J. Leveno, M.L. Sherman,* D.M. Kline,* Dept. Ob/Gyn, Univ. Texas Southwestern Med. Ctr., Dallas, Texas

The impact of chorioamnionitis on the course of labor is controversial. Some clinicians believe chorioamnionitis has stimulatory effects while others suspect inhibitory influences. 266 pregnancies with chorioamnionitis and requiring labor stimulation with oxytocin were matched for age, race, parity, gestational age, oxytocin dosage regimen, cervical dilatation at initiation of oxytocin, and time from rupture of membranes to initiation of labor stimulation.

RESULTS:

OUTCOME	CHORIO-AMNIONITIS BEFORE OXYTOCIN N=69	CASE MATCHED CONTROLS N=69	P	CHORIO-AMNIONITIS AFTER OXYTOCIN N=197	CASE MATCHED CONTROLS N=197	P
C/S (%) Dystocia	6 (9)	6 (9)	NS	78 (40)	20 (10)	<.0001
Fetal Distress	4 (6)	2 (3)	NS	9 (5)	8 (4)	NS
Oxytocin to Delivery Hours, Mean (SE)	4.3 (.2)	5.6 (.4)	.04	12.6 (.4)	7.9 (.4)	<.0001

Conclusions: The impact of chorioamnionitis on the course of labor can be divided into two clinical presentations. Primary chorioamnionitis diagnosed upon admission may enhance labor stimulation and does not increase the risk for cesarean section. However, secondary chorioamnionitis diagnosed after initiation of oxytocin may be a sign of abnormal labor since it is associated with a marked increase in abdominal delivery for dystocia.

234 THE PEDIATRIC GRAVIDA: MATERNAL OUTCOME TB Jones, HM Wolfe, MP Dombrowski, NE Roumayah[x], and SF Bottoms. Wayne State Univ., Hutzel Hospital, Detroit, MI.

Adolescent pregnancy has been associated with increased risk for obstetrical complications such as preeclampsia, operative delivery, low birth weight, and neonatal morbidity and mortality. Little is known, however, regarding the intrapartum course of the pediatric gravida (age ≤14). We compared the labors of 412 pediatric gravidas to 13,222 nulligravidas aged 20-25 delivering from 1983-1990. Odds ratios and 95% confidence intervals are tabulated below.

	Age≤14	Age 20-25	Odds	C.I.
Preeclampsia	7.2%	3.3%	2.3	1.5-3.3
1° Cesarean	14.0%	13.3%	1.0	0.8-1.4
Forceps	5.5%	3.9%	1.4	0.9-2.2
Vacuum	3.9%	0.8%	4.6	2.7-7.9
Episiotomy	64.3%	38.6%	2.8	2.3-3.5
3° or 4° Lac.	20.3%	6.9%	3.4	2.7-4.3

Pediatric gravidas had longer second stage labor (31±31 mins.) than the older group (24±31 mins., $p<0.01$). There was no significant difference in first stage of labor, route of breech delivery, use of classical uterine incisions, or estimated blood loss. Other than increased use of local, anesthesia administration was similar in the two groups. We conclude that pediatric gravidas 1) are more than twice as likely to have preeclampsia, 2) are more likely to undergo operative delivery, but only by the vaginal route, and 3) suffer significant perineal lacerations despite more frequent use of episiotomy.

235 COMPUTERIZED COLPOSCOPY AND CONSERVATIVE MANAGEMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA IN PREGNANCY

Magdy S. Mikhail[x], Irwin R. Merkatz, Akolisa Anyaegbunam, Seymour L Romney[x]. Albert Einstein College of Medicine, Bronx, New York.

Cervical punch biopsies in pregnancy can be associated with excessive bleeding and may induce prostaglandin release which may precipitate premature labor. Objective sequential monitoring of cervical intraepithelial neoplasia (CIN) lesions by a noninvasive technique, to ensure that the lesion is not progressing, may obviate the need for a biopsy. Computerized colposcopy allows for image processing and storage, contrast and edge enhancement, the detection of subtle changes and computer assisted quantification of lesions. Thus, lesion size changes can be objectively demonstrated and monitored. Seventeen pregnant patients with abnormal Pap smears and a fully visualized squamo-columnar junction were serially monitored during their pregnancies using computerized colposcopy. All patients had baseline computer-assisted measurements of their cervical lesions and a repeat measurement at monthly intervals. The mean age of the patients was 27 and the mean size of the lesion was 59 mm^2. During the period of observation 17.7 % of colposcopically visualized lesions increased in size, 11.8 % remained unchanged, 41.1 % decreased in size, and 29.4 % disappeared completely. In patients with an increase in lesion size (n=3) a biopsy was performed which revealed CIN III but no microinvasion. No biopsy was needed in patients whose lesions remained unchanged (n=2), demonstrated a decrease in size (n=7) or disappeared (n=5). Thus cervical biopsy during pregnancy was avoided in 82.3% of cases. At postpartum follow up, all patients had colposcopically directed punch biopsies. Only those patients with an increase in lesion size revealed high grade CIN. This confirms previous observations that lesion size correlate with CIN grade. The ability to sequentially quantitate CIN lesions during pregnancy, using computerized colposcopy, provides an objective noninvasive mode to evaluate progression/regression. Computerized colposcopy has the potential to replace subjective colposcopic evaluation with objective computer assessment and may have a role in the conservative management of CIN in pregnancy.

236 CLINICAL FUNCTIONS OF A MULTI-SITE COMPUTERIZED OBSTETRICAL ULTRASOUND DATABASE. JE Deaver and K Johnson[x]. UTHSC at Houston, Dept Ob, Gyn and Repro Sci, and Comprehensive Informatics for Perinatal Health (CIPHI), Citrus Heights, CA.

Typical obstetrical ultrasound software programs perform data collection and transformation, display gestational age-related plots, and create reports having numerical tables in addition to descriptors merged with static phrases. These databases often have a narrow focus on ultrasound procedures at one site rather than on patient care at many sites. We designed and will demonstrate a computerized ultrasound record accessible from multiple sites designed to integrate ultrasound data with patient-oriented functions. The system is a module of the Athena OMS (CIPHI) computerized prenatal record, uses SQLBase database server (Gupta Technologies), and runs under the Windows 3.0 (Microsoft) graphical user interface on IBM-compatible computers. The resulting software aids in risk assessment (dating, fetal growth restriction, post-dates), allows users to create clinical protocols which, for quality assurance, can be reconciled with orders, and provides reminders concerning diagnoses, risk assessment, data errors, and protocol compliance. Ease of use is accomplished by selection of graphical objects rather than by key entry of commands. In addition to the traditional functions of ultrasound data collection and reporting, the system we describe enhances the clinical utility of ultrasound data.

237 INTERNETWORK COMPUTING FOR PERINATAL RESEARCH: A PROTOTYPE SYSTEM. L.C. Chik,* V. Salari,* R.J. Sokol, Dept. Ob/Gyn, Wayne State Univ./Hutzel Hosp., Detroit, MI

Perinatal computing has increased in breadth and complexity. Typically, applications are mounted on multiple platforms, from pc's to mainframes, limiting access. Thus, for example, in our department, most perinatal computing has been performed on a multi-user minicomputer, leading to excruciatingly slow response times at high load periods, such as the day before the SPO abstract deadline. A plethora of pc's and Macintoshes, as well as 2 Sun workstations, is also available. It would be desirable if a faculty member could execute a stepwise multiple regression, using a program stored in a network host in the perinatal database lab under a network file system (NFS) with the input data file in her study directory. Results could be sent immediately to her colleague at another host computer. Such an information processing scenario was foreseen a decade ago when DARPA (Defence Advanced Research Projects Agency) supported the development of TCP/IP (Transmission Control Protocol/Internet Protocol) in Ethernet networks for heterogeneous computers. With a contemporary NFS, we have implemented up-to-date internetwork capacity with a departmental database and a research host computer serving a consortium of investigators and mixed personal computers. This has resolved serious conflicts between acute heavy research demand and daily clinical database operations. As a benchmark, a Sun workstation can be accessed from multiple sites to perform large statistical analyses at > 20x the previous speed of the minicomputer, now used primarily for clinical database operation. Likewise, we can utilize network resources from different computers to enter a patient dataset in the ultrasound laboratory, generate a report on the database host and combine an alphanumeric report with a gray scale ultrasound image on the departmental letter head for a referring physician. Internetwork technology has great potential for clinical application for functional quality assurance and decision support.

238 PERI: A COMPUTER INFORMATION SYSTEM FOR THE PRACTICE OF PERINATAL MEDICINE. Robert N. Wolfson, M.D./Ph.D., Damon Stafford[x], President, Memorial Hospital, Colorado Springs, CO, and LewMin Inc., Albuquerque, NM

Perinatal medicine is characterized by a broad range of services that may include perinatal consultation, genetic consultation, antepartum testing, diagnostic ultrasound, prematurity prevention and specialized procedures such as amniocentesis, CVS and PUBS. In any one pregnancy patients may utilize only one or perhaps all of the services offered. The purpose of this project was to develop a computerized uniform chart for tracking, scheduling and reporting of services. PERI is the result of a joint effort of a Perinatal Unit and software developer to achieve a computer information system designed for Perinatal Medicine. Using a multi-user/multi-tasking computer system, PERI has customized data entry to achieve perinatal, genetic and prematurity prevention consultation as well as progress notes and other annotations. NST monitor data is obtained in realtime to produce a report including a compressed graphical presentation of the NST as well as other interpretive data. Ultrasound reports are derived from concurrent data entry during examination and yield tabular and graphical presentation of the findings along with interpretive dialogue and clinical management considerations. Preliminary experience with PERI suggests that it fulfills the diverse needs of the Perinatal practice. The challenge, benefits and obstacles of developing new computer software will be presented.

239 AN AUTOMATED OBSTETRICAL DATABASE THAT DOES MORE. J. P. VanDorsten, T.C.C. Peng, P. Dilzer[x], Medical College of Virginia-Virginia Commonwealth University, Richmond, VA.

Since October, 1990 the Division of Maternal-Fetal Medicine has successfully integrated into the Hospital Information System's (HIS) main frame computer an automated obstetrical database which generates ultrasound reports, antepartum testing reports, admission history and physicals, labor and delivery summaries, discharge summaries, and birth certificates. The advantages of this user interactive system are as follows: 1. on-line entry utilizing light pens and menus at one of 350 terminals, 2. step-wise entry by physicians and nurses to minimize entry time (≤ 5 minutes for any one data set), 3. immediate availability of data at any terminal, 4. legible, timely, and accurate reports, and 5. elimination of redundant data entry by healthcare providers. In this computerized obstetrical database, many additional tasks are expedited: 1. monthly quality assurance reports using ACOG obstetrical indicators, 2. maintenance of housestaff experience logs as required by the Residency Review Committee, 3. maintenance of patient lists for attending physicians as required by the ABOG, 4. ad hoc reports, queries, and clinical research, and 5. documentation of utilization of labor and delivery services and referral patterns. In summary, this automated database fulfills many non-traditional roles that improve the quality and efficiency of health care.

240 PATIENT LIST SOFTWARE FOR CANDIDATES TAKING THE ORAL EXAM IN MATERNAL-FETAL MEDICINE
C. Stedman and A.G. Robichaux III, Dept. Ob/Gyn, Ochsner Clinic, New Orleans, LA

Although software exists to compile case lists for the general oral examination in obstetrics and gynecology, no application is offered nationally for the maternal-fetal medicine (MFM) case list. As of 1990 the composition of the MFM summary form changed dramatically from that required for the general exam, and a tallying of 48 patient subgroups is now mandated. We wish to report a FileMaker Pro® (Claris, Santa Clara, CA) template capable of generating the obstetrical, gynecologic, and summary forms specific for the MFM lists. This program functions with Macintosh SE, SE/30, IIcx, IICi, IIfx, IIsi, Classic, or LC models. System software may be 6.05, 6.07, or the recently introduced system 7.0. We have created a work sheet that allows tabulation of all required data so the physician is spared subsequently recalling the chart from medical records. The typical work sheet requires just 2-3 minutes to complete. Modules are available in either black/white or color; users may choose data entry screens customized for either 13", 16", or 19" monitors. Buttons and macros allow easy report generation by a laser printer. Cases in which the physician acted solely as a consultant for inpatients are listed separately as requested by the American Board of Obstetrics and Gynecology.

241 FETAL IMAGING WORKSTATION. W. Lee*, C.H. Comstock*, J.S. Kirk, Div of Fetal Imaging, William Beaumont Hospital, Royal Oak, MI; Wayne State University *

An interactive fetal imaging computer workstation has been established (William Beaumont Hospital Research Institute, March of Dimes Birth Defects Foundation, Apple Computer) to examine various ways that images and sophisticated computer graphics can be used to interactively teach physicians about the prenatal diagnosis of birth defects. Main features include: 1) fetal ultrasound image and animation library; 2) medical image archival system; 3) data-base categorization and image search capabilities; 4) interactive multimedia tutorials; and 5) in-house slide production. This system uses a Macintosh IIfx computer with a 19 inch Supermac monitor. Ultrasound images are typically captured from videotape (Nuvista card, FORA color encoder, FORA time-based corrector) or photographs (Sharp JX-600 scanner). Correlative autopsy specimens are either digitized from standard photographs or by an electronic still camera system (Sony ProMavica). These images are postprocessed through Adobe Photoshop and digitally archived onto optical disk. Processed images can be placed onto hard disk, laserdisc (Panasonic TQ-3031F), or CD-ROM for inclusion into a variety of educational tutorials via Macromind Director. A Supercard application provides clinical database organization and search capabilities for up to 108,000 images on a 12-inch laserdisc. We are currently in the process of developing interactive multimedia software interfaces for the fetal imaging library. Photorealistic 3-D animation software will be eventually used to simulate normal and abnormal fetal heart development. Our works-in-progress demonstration will show potential uses for this visually-based information management system.

242 CUSTOMISED ANTENATAL GROWTH CHARTS

J Gardosi[x], A Chang[x], B Kalyan[x], EM Symonds[x]
Perinatal Research & Monitoring Unit, Dept Ob/Gyn
Queens Medical Centre, Nottingham, England

A new antenatal chart was developed which can be 'customised' to each mother's individual characteristics and used to assess birth centiles of her previous babies, as well as fundal height measurements and ultrasound - estimated fetal weights during the ongoing pregnancy. Multiple regression and variance analysis was performed on computerised data from 4179 pregnancies with ultrasound-confirmed dates. This showed that in addition to gestation and sex, the maternal booking weight, height, ethnic group and parity were significant, independent determinants of birthweight in our population. Correction factors were calculated and entered into a computer programme which uses this database to generate a personalised growth chart with corrected centile curves. This can be printed out at the beginning of pregnancy and added to the notes. Such adjustment of normal limits resulted, in our retrospective sample, in a reduction of false-positive and false-negative diagnoses of 'SGA' (small for gestational age; <10th centile) by 28% and 24% , and 'LGA' (large for gestational age; >90th centile) by 22% and 26%, respectively. We suggest that the relevant physiological variables which act as determinants of birthweight in a particular maternity population need to be better recognised in clinical practice. Prospective analysis is planned, and we hypothesize that such 'customisation' of growth assessment will reduce unnecessary investigations, interventions and patient anxiety and lead to better allocation of existing resources.

243 A COMPREHENSIVE PERINATAL DATABASE PROGRAM. P. Urso,[X] J.A. López-Zeno,[X] B. Nies,[X] S. Bathgate,[X] J. Grossman. Dept. of OB-GYN, The George Washington University Medical School. Washington, D.C.

Using a relational database program (OMNIS) we have designed a comprehensive perinatal database. It is capable of storing all the information that is requested in the ACOG Prenatal Forms This includes all data from prenatal visits, including laboratory test results. In addition, it is capable of storing and generating fetal surveillance reports, like NST. CST or BPP. Calculations of gestational age and fetal weight are incorporated into the ultrasound reports generated by this database. Upon admission to labor and delivery, all outpatient information is available in a computer terminal. The physician's admission, delivery and discharge notes can be computer generated with the information collected during labor and delivery. Nursing notes and birth certificates are also incorporated into the database. Our system provides a single, unified data resource capable of meeting both research and quality assurance needs. At our institution, the peer review process is currently conducted with information primarily obtained from this database, which conforms to the JCAH obstetrical standards. It also generates resident's experience forms and attending's case list for the ABOG. We are currently integrating the obstetrical anesthesia and neonatal data into this program. This program can be utilized in the Macintosh or the IBM operative systems. A demonstration of the Macintosh version will be given at the SPO meeting.

Poster Session III

Friday, February 7, 1992
10:00 a.m.-12:30 p.m.

Grand Salons I-IV

CATEGORIES	POSTER NOS.
Genetics and Teratology	244-285
Fetal Therapy	286-293
Prematurity	294-329
Placental Physiology	330-343

244 **SIMILARITY OF TWINS TO SINGLETON MSAFP RATIO BY RACE: NO NEED TO ESTABLISH SPECIFIC MULTIFETAL TABLES.**

A Drugan,x JE O'Brien, R Gambino, MP Johnson,* MI Evans, Depts Ob/Gyn, Hutzel HospitalWayne State U, Detroit, M , Rambam Medical Center, Haifa, Israel, and MetPath Inc., Teterboro, NJ

MSAFP values in 535 twin gestations were grouped according to week of gestation (15-20 wks) and race. MSAFP (MIU/ml) in blacks was higher than in whites (p<.01).

	White Pregnancies				Black Pregnancies			
GA	No.	Twins	Single	Ratio	No.	Twins	Single	Ratio
15	81	56.8	26.1	2.17	19	56.3	25.8	2.18
16	170	60.6	28.3	2.14	33	77.6	30.8	2.52
17	101	69.9	32.3	2.16	25	83.4	33.2	2.51
18	50	74.7	38.0	1.97	18	85.1	45.1	1.96
19	16	83.2	43.1	1.93	6	88.0	48.1	1.83
20	10	107.5	44.4	2.42	6	96.5	56.7	1.70
Total	428			2.13	107			2.10

Using race specific cutoffs of 4.5 MOM, 3.7% of whites and 4.7% of blacks were considered high, which is comparable to findings in singletons. These differences would not have been found in a non-adjusted mixed race database. "Low" results (1.0 MOM for age-matched singletons) were seen in 7.2% of whites and 5.6% of blacks, comparable to singleton pregnancies. Using race-specific databases, the interracial differences in frequency of either "high" or "low" results was not significant. We conclude: 1) different databases should be used in black and white twin pregnancies, 2) "High" (>4.5 MOM) and "low" (<1 MOM) cutoff values derived from the large databank of singleton pregnancies seem to be adequate for interpretation of MSAFP results in twins, obviating the need to build specific median curves for multifetal gestations.

245 FETAL CHOROID PLEXUS CYSTS: THE SMALL OR TRANSIENT CYST IS NOT NECESSARILY BENIGN. V. Klein, M. Perpignano, H. Cohen, F. Mandel, J. Streltzhoff, F. Chervenak, Depts. Ob/Gyn, Radiology and Research, North Shore Univ. Hosp. and NY Hosp., Cornell Univ. Med. College, NY

Analysis of 3770 obstetrical patients having prenatal sonograms between 14 and 32 wks. gestation revealed that 87(2.3%) were found to have fetal choroid plexus cysts. Eighty-three patients underwent amniocentesis, six(7.2%) had abnormal karyotypes. Four cases had the commonly associated chromosomal abnormality, trisomy 18. Two cases had a karyotype not usually associated with choroid plexus cysts, mosaic Turner's and trisomy 21. Of the six abnormal karyotypes, one had a 4 mm unilateral cyst, and 3 had bilateral cysts of 3-5 mm; two had larger cysts. In only one case with a 16 mm cyst had any associated structural abnormalities been detected sonographically. In the two cases of trisomy 18 that were not terminated and in the fetuses with trisomy 21 and mosaic Turner, cysts resolved between 23-26 wks. Our data suggests that fetal karyotyping should be considered in all fetuses with choroid plexus cysts, irregardless of laterality, size or spontaneous resolution prior to birth.

246 IDENTIFICATION OF TURNER SYNDROME WITH FETAL HYDROPS IN MULTIPLE MARKER SCREENING FOR DOWN SYNDROME D N Saller Jr[1], M G Blitzer[2x], S Schwartz[2x] & J A Canick[1x] [1]Brown Univ./Women & Infants Hosp. Prov.,RI & [2]Div. of Human Genetics, Univ. of Md. at Baltimore, MD

We previously reported the identification of non-immune fetal hydrops, some cases of which had Turner syndrome, in multiple marker screening for Down syndrome (which is based on age, low alpha-fetoprotein [AFP], low unconjugated estriol [uE3] & elevated human chorionic gonadotropin [hCG]). These cases were detected because of consistently low uE3 & markedly elevated hCG. In order to investigate the levels of uE3, hCG, & AFP associated with Turner syndrome, we identified 15 cases of 45,X karyotypes (7 with hydrops and 8 without) for which a second trimester maternal serum sample was available. The AFP levels were reduced in both groups (median MoM=0.81, range=0.39-1.59), although 4 of these cases had been referred for low AFP (possibly biasing the AFP levels). The uE3 levels were substantially reduced in both groups (median MoM=0.48, range=0.12-1.45). The hCG levels had a bimodal distribution with no overlap (rank sum test, p=0.001), in which all 7 hydropic pregnancies had markedly elevated levels (median MoM=3.84, range=1.80-5.77) and all 8 non-hydropic pregnancies had low levels (median MoM=0.52, range=0.21-0.76). All of the 7 hydropic cases would have been identified as screen positive for increased Down syndrome risk while none of the 8 non-hydropic cases would have been so identified. These findings indicate that the morpholologic defect of hydrops fetalis, rather than the 45,X karyotype itself, is responsible for the high risk pattern in multiple marker screening for Down syndrome. Non-hydropic 45,X pregnancies have relatively low AFP, uE3 & hCG, and are unlikely to be identified as high risk for Down syndrome. They may, however, be detected as high risk for Trisomy 18 (in which all 3 markers are consistently low).

247 PRENATAL ALCOHOL EXPOSURE AND NEUROBEHAVIORAL FUNCTION IN INFANCY: EVIDENCE FOR THRESHOLD AND DIFFERENTIAL VULNERABILITY. J.L. Jacobson,* S.W. Jacobson,* R.J. Sokol, S.S. Martier,* J.W. Ager,* Depts Ob/Gyn and Psychology and Fetal Alcohol Research Center, Wayne State Univ., Detroit, MI

The relation of prenatal alcohol exposure to anatomic alcohol-related birth defects (arbd) has been characterized in our studies as exhibiting a threshold and differential fetal vulnerability. Though relations of prenatal exposure to neurobehavioral abnormality have been reported, threshold effects and differential vulnerability have not been systematically sought. In a prospectively studied disadvantaged inner city black sample (n=389), periconceptional and antepartum fetal alcohol exposure was estimated from repeated maternal reports and the Bayley scales of infant development administered to offspring at age 1 year by examiners blinded to prenatal history. Consistent with previous studies, after control for potential confounders, higher levels of drinking at conception and during pregnancy were associated with poorer performance on the Bayley with no evidence of threshold. However, when 1 standard deviation below the sample mean was used as the criterion for poor performance, no effect was detectable below 14 oz of absolute alcohol per week (AA/W) (28 standard drinks/w) periconceptionally or 3.5 oz AA/W (7 drinks/w) during pregnancy. Using this criterion, neurobehavioral function was notably impaired in 30% of the higher exposed offspring, but not in the remaining 70%. These results suggest that, as with anatomic arbd, relationships of prenatal alcohol exposure to neurobehavioral abnormality may be characterized by a threshold above which there are marked individual differences in vulnerability to the effects of prenatal alcohol exposure.

248 ANEUPLOIDY IN SECOND TRIMESTER OLIGOHYDRAMNIOS
J. Manley M.D[X], L. Vought, M.S.[X], Stuart Weiner, M.D. Dept. of Maternal - Fetal Medicine, Pennsylvania Hospital, Philadelphia, PA
The diagnosis of oligohydramnios was made in 49 pregnancies in the second trimester between January, 1989 and August, 1991. In all patients, the diagnosis of membrane rupture and suspected twin-twin transfusion was ruled out. Oligohydramnios was suspected subjectively by the examiner, and the amniotic fluid index fell more than one standard deviation below the mean for gestational age. The mean AFI was 3.6. A genetic procedure was offered in all cases, and karyotype was obtained in 46 (94%). One patient declined and cultures failed to grow in two patients.
Aneuploidy was diagnosed in 11 (24%). There were four cases of triploidy, one XYY, two of autosomal trisomy, and four unbalanced translocations. Aneuploid fetuses had a mean AFI of 3.3 (range 0-6) and a mean gestational age at diagnosis of 20.7 weeks. Although ultrasound showed associated abnormalities (growth retardation, elevated head to body ratio, etc). in all but one fetus, the constellation of findings in each case was not specific for the type of aneuploidy. In addition some fetuses with structural abnormalities had normal karyotypes. Of those with normal karytotypes, 37% selectively terminated the pregnancy before viability. The mean AFI in this group was 3.7, and the mean gestational age was 21.2 weeks. Twelve of forty six (26%) survived; all had normal karyotypes, ten had normal ultrasound examinations, one had evidence of distal urinary tract obstruction, and one had multiple, large placental lucencies. the mean AFI was 5.3 and the mean gestational age at diagnosis was 22.5 weeks. This series suggests that not all cases of second trimester oligohydramnios are associated with poor outcome. However, the high incidence of aneuploidy suggests that a procedure for determining fetal karyotype should be offered to provide additional information for management of pregnancy.

249 **EARLY ALTERATIONS IN GAMMA AMINOBUTYRIC ACID (GABA) LEVELS IN FETAL ALCOHOL EXPOSED MICE.** C.S. Zajac[X]. Dept. of Ob/Gyn, Wayne State Univ., Detroit, MI.
Children with alcohol related birth defects (ARBD) exhibit sensory and behavioral deficits which may be associated with an inability to integrate and respond appropriately to external stimuli. There is also considerable behavioral evidence of developmental delay in central nervous system development in these children. The superior colliculus is a sensory integrative area of the brain which plays a major role in coordinating reflex responses to visual, auditory, vestibular and somesthetic input. Altered levels of the inhibitory neurotransmitter gamma aminobutyric acid (GABA) in the superior colliculus may contribute to the attentional and behavioral problems of children with (ARBD). Alterations in the developmental expression of GABA by prenatal alcohol exposure may affect an individual's capacity for dealing with excessive afferent input to the brain. Such individuals would be unable to screen out excess sensory stimuli and would exhibit sensory and behavioral deficits such as attention deficits. Using a fluorometric microassay of lyophilized brain tissues, levels of GABA were determined in the superior colliculus of Swiss Webster mouse fetuses (gestation day 15) which had been exposed prenatally to 3g/kg ethanol/day by gastric intubation. A nutritional control group was intubated with maltose dextrin solution isocaloric to the alcohol. Significantly higher levels of GABA were found in the superior colliculus of gestation day (GD) 15 mouse fetuses exposed to alcohol in utero when compared with control animals. Previous studies have shown increased neuronal nuclear density (i.e. increased number of neurons) in the same area of the brain in GD 18 mice. Since neurons are overproduced in normal development, and then decrease in number as normal synaptogenesis occurs, it is hypothesized that the increased level of GABA at GD 15 may reflect developmental delay as well. This may provide insight into possible pharmacologic amelioration of some of the problems of the fetal alcohol exposed child. (Supported by grant P50 AA07606 from NIAAA).

250 **EAR LENGTH IN SECOND TRIMESTER ANEUPLOID FETUSES.** Luanna Lettieri, John F. Rodis, James F.X. Egan, Anthony Vintzileos, Lori Feeney[X], Patricia Dellaripa[X], University of Connecticut Health Center, Farmington, CT
Abnormally small ears have been noted to be one of the most consistent clinical findings in patients with Down syndrome and other aneuploid conditions. We undertook a study to test the hypothesis that abnormally short ears are of diagnostic value in detecting second trimester aneuploid fetuses by ultrasound. We prospectively studied 326 consecutive patients undergoing ultrasound examination for genetic amniocentesis from 14-22 weeks gestation with singleton pregnancies from January - July 1991. The standard fetal biometry measurements were obtained, including ear length (from helix to the tip of the lobe). Of these patients, 87% (283) had ear measurements obtained and a nomogram for ear length by gestational age was compiled. The ear lengths of the remaining 13% (43) were not obtained due to unfavorable fetal position. The relationship between the ear length and gestational age was linear ($R=.7$, $p<.001$). Scattergrams were developed and regression analyses were used to establish the 5th, 10th, 20th, 50th, 80th, 90th and 95th percentile lines. Six fetuses were found to have a chromosomal abnormality: 4 had trisomy 21, one had trisomy 18 and one was a mosaic trisomy 21. All six aneuploid fetuses had ear lengths $\leq$ 20%ile for gestational age for a sensitivity of 100%. The specificity, positive and negative predictive values were 71% (196/277), 7% (6/87) and 100% (196/196), respectively. Using a cutoff of $\leq$ 10%ile for gestational age, the sensitivity was 67% (4/6) with a specificity of 82% (226/277). The positive and negative predictive values were 7% (4/55) and 99% (226/228), respectively. The trisomy 18 fetus had an ear length below the 1% for gestational age. We suggest that fetal ear length may be useful in sonographically identifying aneuploid fetuses in the second trimester.

251 **CONTINUUM OF GENOMICALLY ALTERED GROWTH IN MONOSOMY, DISOMY, & TRISOMY 21: OBSERVATIONS FROM THE FIRST PRENATALLY DIAGNOSED MONOSOMY 21.** Mark Paul Johnson[X], Philip Arbit[X], John Lusak[X], Faisal Qureshi[X], Nelson B. Isada, Peter G. Pryde[X], Mark I. Evans, Depts. OB/GYN & Pathology, Hutzel Hospital/Wayne State University, Detroit, MI.
We have previously investigated the differental patterns of organ-specific intrauterine growth retardation in trisomies 18, 21, and monosomy X. We showed that IUGR patterns are chromosome specific and established gravimetric indices for the major organ systems. Utilizing this approach, we have examined the first case of prenatally diagnosed monosomy 21, and observations raise the possibility of a numerically influenced growth potential for chromosome 21. Direct necropsy measurements of body and visceral weights from the 22 wks. gestation monosomy 21 fetus were compared to expected weights based on power equation body-wt. indices.

Organ	Monosomy 21	Disomy 21	Trisomy 21
adrenal	3.3 gms	1.25 gms	0.48 gms
brain	60.0	44.05	42.40
heart	3.5	2.09	1.36
kidney	10.0	2.42	1.45
liver	macerated	15.41	15.45
lung	4.80	4.45	3.96
thymus	macerated	0.46	0.06
body wt.	295.00	(460.00)	283.30

Overall body weight was decreased isecondary to the presence of caudal regression sequence with markedly shortened lower limbs. However, organ weights were much higher than expected which implies a more rapid growth rate in this fetus. When the three groups are compared, there appears to be a numeric-specific growth potential continuum, inversly based on the number of chromosome 21s present. Therefirem normal growth may be dependent on a disomic 21 state, while growth is depressed in trisomic states and accelerated in monosomic states. We believe this is the first demonstration of a possible relationship of chromosome number to fetal growth rates, and implies the presence of fetal growth regulatory systems associated with chromosome 21.

252 **ACE INHIBITOR FETOPATHY.** Peter G Pryde,* Clark E Nugent, Aileen B Sedman,* Mason Barr Jr.* Depts of Ob/Gyn, Pathology, and Pediatrics, University of Michigan Medical Center, Ann Arbor, MI and Dept Ob/Gyn, Division of Repro Genetics, Hutzel Hospital/Wayne State U, Detroit, MI.

The ACE-inhibitors (AI) are widely prescribed and effective antihypertensives, but are not without risk in pregnant women. Profound fetal toxicity has been reported in several animal models. Adverse human-fetal outcomes have also been reported. We describe a pattern of anomalies and physiologic alteration which can be termed AI fetopathy and report three additional infants in which this peculiar pattern is manifest (table).

Case	Maternal disease, medication	Gestation,mode of delivery, birth wt (%-ile)	Oligo	Neonatal hypoten-sion, anuria	Other
#1	26 y/o, 8 yr SLE, chronic HTN, on captopril	33 wk, fetal distress, induction, vaginal breech, 1440 g male (< 2.5%-ile)	severe	profound unremitting	Potters features, IUGR, PH, HC, RTD, death @ 14 hrs.
#2	15 y/o, 10 yrs HTN, on lisinopril	32 wks, fetal distress, cesarean section, 1480 g male (< 2.5%-ile)	severe	profound (improved only after dialysis)	IUGR, HC, RTD and ATN, persistant renal insuffiency, on dialysis, now 15 mo of age.
#3	30 y/o, 9 yrs chronic HTN, on enalopril	32 wks, fetal distress, cesarean section, 980 g male (< 2.5%-ile)	severe	profound (improved on dialysis)	IUGR, HC, short limbs, RTD, death @ 9 de of life (renal failure)

These cases combined with those previously reported indicate that AIs are fetotoxic. The peculiar pathophysiologic pattern cannot be ascribed to the underlying materna disease or other medications. AI fetopathy is characterized by fetal hypotension, anuria-oligohydramnios, growth-restriction (IUGR), hypocalvaria (HC), renal tubular dysplasia (RTD), and in the most severe cases pulmonary hypoplasia (PH). In surviving neonates profound hypotension and anuria are observed which are recalcitrant to volume replacement and pressor therapy. The hypotension resolves only after dialysing off the otherwise renally secreted drug. Although present data do not allow inference of frequency of AI fetopathy in exposed pregnancies, these drugs should be viewed as human fetotoxins and considered only as a last resort for use during pregnancy.

253 **FAMILIAL OMPHALOCELE (FO): CONSIDERATIONS IN GENETIC COUNSELING.** PG Pryde,* NB Isada, MP Johnson*, MI Evans, Dept OB/GYN, Hutzel Hosp/Wayne State Univ, Detroit, MI

Isolated, nonsyndromic omphalocele is generally regarded as a sporadic malformation. Recurrence risks (RR) are considered negligible. Our observations on a patient in whom five consecutive pregnancies (by 2 separate nonconsanguenous partners) were complicated by FO (see table) suggests a need to alter the standard counseling for RR. Neither the patient nor her partners had history of relatives affected by omphalocele although the patients brother and his son had very large umbilical hernias requiring surgical repair. Some cases of familial isolated omphalocele have been reported. Most pedigrees are suggestive of a vertical mode of transmision although there are a few cases with only a single generation involved. In no previously reported case was a complete generation, and so many members affected. It is problematic to speculate as to the genetic mechanism operating in this family.

Preg.#	Karyotype	Delivery	Outcome
1	not done	25 wks SVD	680 g F, omph, death @ 24 h
2	46,xx	16 wks SAb	ultrasound confirmation omph prior to Ab
3	not done	29 wks labor, classical CS	1500g M, omph, repair, death @ 5 mo
4	not done	30 wks labor, LTCS (repeat)	1590 g M, omph, repair, death@ 24h
5	46,xx	30+ wks labor,LTCS (repeat	1650 g F, omph, repair, death@ 24 h

Abbreviations: omph= isolated omphalocele, M= male, F= female, SAb= spontaneous abortion, LTCS= low transverse cesarean section, SVD= spontaneous vaginal delivery

Because the defect occurred with two separate fathers a monogenic autosomal recessive mechanism is unlikely. However, the finding of a large umbilical hernia in the patient's brother and his son, makes an autosomal dominant mechanism with variable expressivity a tenable explanation. A polygenic-multifactorial mechanism which might be suggested as an alternative explanation is not satisfying in light of the 100% transmission through 5 siblings. With present data and diagnostic limitations the true mechanism operating cannot be elucidated. Undoubtedly it is largely genetic. These cases emphasize omphalocele heterogeneity and caution in counseling RR.

254 **DETERMINANTS FOR PARENTAL DECISION TO ABORT (DTA) OR CONTINUE FOR NON-ANEUPLOID ULTRASOUND DETECTED ABNORMALTIES.** PG Pryde,* AE Odgers,* NB Isada, MP Johnson, MI Evans. Dept OB/GYN, Reprod. Genetics, Hutzel Hospital/Wayne State U, Detroit, MI.

Decision to abort an otherwise wanted pregnancy because of fetal anomalies is complex. This study evaluates DTA after finding malformations on ultrasound in the karyotypically normal fetus. All pregnancies managed on our service complicated by ultrasound abnormalities from 4/90-8/91 were included (n=262). Cases with associated karyotypic abnormalities (KA) were excluded (n=35) as were cases in which a diagnosis was made after the legal gestational age (GA) limit for abortion (24 wks, n=65). The remaining 159 cases were stratified into prognosis groups of severe, uncertain, and mild. Data were analyzed using ANOVA with decision to continue (C) or terminate (T) as the dependent variable. **Results:** Mothers age, gravity, parity, and gestational age at diagnosis were not significantly different between groups. Severity of the ultrasound abnormality was strongly correlated with DTA (p=.0001).

	Mild	Uncertain	Severe	Totals
T	2.8%	11.1%	65.6%	30.2%
C	97.1%	88.9%	34..4%	69.8%
Total	100%	100%	100%	100%

Conclusions: 1. In non-aneuploid pregnancies with ultrasound diagnosis of fetal abnormality, the major predictor of DTA is severity of prognosis. 2. Contrary to previous assertions by other authors, but in agreement with our previous study in KA fetuses, the GA at the time of diagnosis is not an important variable in DTA. 3. Contrary to widely held opinion, most parents having fetuses with significant anomalies which carry uncertain prognoses opted to continue pregnancy. This was particularly true for defects potentially correctable by in-utero intervention (eg. bladder shunt, data not shown).

255 **ANTENATAL DIAGNOSIS USING AMPLIFICATION OF FETAL DNA FROM MATERNAL BLOOD.**

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To determine the practicality of testing fetal cells present in the maternal circulation for genetic diagnosis, a model testing for the Y chromosome was selected. 50 maternal blood samples were obtained from gestations ranging from 6 weeks to term. DNA was extracted from the samples. The polymerase chain reaction was used to search for Y-specific sequences (of fetal origin) using either nested primers (Lo et al, *Lancet* 1990) which detect a single copy region or primers to Y alphoid repeats (Witt et al., *Hum Genet* 1989). Results were compared to the neonatal sex recorded at delivery . Sensitivity (ability to correctly identify male fetuses) was 80-100%, increasing with each successive trimester of pregnancy. Specificity varied from 20-92% depending on the primer set and the assay conditions. False positives occurred predominantly with samples from multiparous patients who had previously delivered a male child. This model suggests that amplification of paternal specific markers from maternal blood could reduce the need for invasive diagnostic procedures in order to perform DNA testing, especially in nulliparous gestations.

256 **ASSESSMENT OF THE INFLUENCE OF CHERNOBYL ON BIRTH DEFECTS AND ABORTION RATES IN AUSTRIA.**

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The teratogenic potential of low dose radiation is difficult to determine. The purpose of this study was to assess whether measured radioactive fallout (predominantly I^{131} or Cs^{137}) following the Chernobyl disaster in April 1986 altered the rate, pattern or regional distribution of birth defects (BD) or abortion rates in southern Austria. The Styrian malformation register (SMR) was set up as a population-based regional multi-source system whereas the existing government register still relies on one source and therefore provides poor ascertainment especially in cases of internal defects (overall BD rates: 2.3% vs 1%). Over a period of 5 years (1985-89) the SMR monitored 66,740 total births. 64 sources provided data on 1695 cases of BD, of which 1617 were suitable for analysis. All cases were analysed in terms of their calculated conception date and coded (ICD9) and divided into 3 main groups, based on their vulnerable phase of embryogenesis: Group I (n=157): cyclopy, NTD, esophageal atresia, etc. Group II (n=630): CHD, facial clefting, diaphragmatic hernia, syndactyly, etc. Group III (n=133): de novo chromosomal anomalies. In group III the vulnerable time of spermato- and oogenesis was considered. To study possible regional clustering, the pre- and post-Chernobyl rates were plotted in 17 subdistricts. No significant changes in incidence or regional distribution of BD were observed, but a baseline BD rate has been established for future surveillance.

To study other possible effects of the disaster, the overall abortion rate and the counselling frequency at termination clinics for periods of 1.5 years before and after the event were compared. All Styrian pathology departments provided data on 7775 abortions (16% of 48,017 total births). The abortion rate and the counselling frequency was not been altered significantly following the disaster.

257 **UNEXPLAINED ELEVATED SECOND TRIMESTER MATERNAL SERUM ALPHA FETOPROTEIN: A MARKER FOR LOW BIRTH WEIGHT.**

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The association between elevated maternal serum alpha fetoprotein (MSAFP) values and fetal anomalies, multiple gestations and intrauterine demises has been well described. However, the significance of unexplained elevated MSAFP values remains unclear as does the appropriate subsequent pregnancy management for these women. The purpose of our study was to prospectively follow those women with unexplained elevated MSAFP values throughout their pregnancies. From the beginning of our MSAFP screening program in March 1988 until February 1991, 12,530 women in our single center, indigent population underwent MSAFP sampling between 15 and 19 weeks of gestation. Of these women, 259 had elevated MSAFP values (greater than or equal to 2.5 MoM). 51 women (19%) had unexplained elevated MSAFP values after repeat blood sampling and high resolution ultrasound, with or without amniocentesis for amniotic fluid AFP, acetylcholinesterase, and fetal karyotype. These women were subsequently followed with frequent clinic visits and serial ultrasound examinations for fetal growth. There were two spontaneous abortions and 49 live born infants, 13 (27%) of which were low birth weight (less than 2500 grams). This differs significantly from the rate in our general obstetric population (10%, $P<0.001$) during this time. We conclude that in our population, unexplained elevated MSAFP values are associated with an increased incidence of low birth weight infants, the cause of which remains unclear.

258 ABNORMAL SECOND TRIMESTER ULTRASOUNDS ARE ASSOCIATED WITH KARYOTYPIC ABNORMALITIES.

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Anecdotally, it is evident that fetal aneuploidy is associated with abnormalities of amniotic fluid volume (AFV) and fetal structural anomalies, however, the risks have not been determined. We reviewed prospectively collected data from 2822 second trimester ultrasounds and karyotypes in a high-risk, referral population. Abnormal AFVs were diagnosed by subjective and objective criteria. The index-risk sample included gravidas <35y with structurally normal fetuses and normal AFV. Odds ratios and 95% confidence limits (CL) for abnormal karyotypes among structurally normal fetuses with abnormal AFV are listed below:

Decreased AFV	4 of 63	5.0 (CL 1.7 to 15.2)
Oligohydramnios	0 of 28	
Decreased + Oligo	4 of 91	3.4 (CL 1.1 to 10.2)
Increased AFV	3 of 26	9.7 (CL 2.7 to 35.0)
Polyhydramnios	1 of 17	4.6 (CL 0.6 to 36.8)
Increased + Poly	4 of 43	7.6 (CL 2.5 to 23.4)

The risk of abnormal karyotype was greater among all cases of abnormal AFV (8 of 134) than age >35y (16 of 1027); 4.0 (CL 1.7 to 9.6). Structurally abnormal fetuses with normal AFV were at markedly increased risk for abnormal karyotypes (16 of 116); 11.9 (CL 5.9 to 23.7). We conclude: 1) 2nd trimester abnormalities of AFV, whether increased or decreased, have a significantly increased risk of abnormal karyotypes, 2) such patients should be offered karyotypes, 3) structural anomalies, even with NAFV, are associated with a markedly increased risk of abnormal karyotype.

259 **AMNIOTIC FLUID ACETYLCHOLINESTERASE (ACHE) IS FOUND WITH GASTROSCHISIS BUT NOT OMPHALOCELE.**

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Amniotic fluid ACHE has been used to assist in the evaluation of neural tube defects. It has also been detected in ventral wall defects. We examined amniotic fluid ACHE in 24 pregnancies, 16 complicated by gastroschisis and 8 by omphalocele. One omphalocele was ruptured antenatally and was excluded. In 22 out of 23 cases, a normal karyotype was found; one omphalocele had trisomy 13. ACHE was measured by polyacrylamide gel electrophoresis as previously reported and results reported as positive, suspicious, or negative. Results shown below were analyzed by chi-square.

ACHE	Omphalocele	Gastroschisis
Positive	0	12
Negative	7	0
Suspicious	0	4

$\chi^2=.0001$

All omphaloceles were negative for ACHE, while all gastroschises were positive or suspicious. We conclude that a positive or suspicious ACHE is highly associated with gastroschisis or a ruptured omphalocele, but not with an intact omphalocele. Such findings are consistent with the differing origins of the defects and may be used in the differential diagnosis.

260 **SIGNIFICANCE OF AMNIOTIC FLUID PLATELET FACTOR 4 AND BETA-THROMBOGLOBULIN IN GENETIC AMNIOCENTESIS.** AA Saleh, NB Isada, MP Johnson, MI Evans, T Ozawa, MP Dombrowski, M Treadwell, EF Mammen, Dept Ob/Gyn, and Center for Fetal Diagnosis and Therapy, Hutzel HospitalWayne State University, Detroit, MI.

Platelet factor 4 (PF4) and betathromboglobulin (BTG), unique markers of irreversible platelet activation, have not been evaluated in amniotic fluid. While PF4 is mainly cleared by endothelium and to a lesser extent by kidneys, BTG is only cleared by kidneys. Therefore, amniotic fluid PF4 and BTG levels may reflect fetal platelet activation, endothelial and/or renal function. We measured PF4 and BTG by ELISA in amniotic fluid from 78 patients with normal α-fetoprotein (AFP) and 24 with high AFP (anencephaly 5, gastroschisis 5, fetal demise 8, cystic hygroma 1, placental hematoma 1, hydrocephalus 4). All pregnancies ranged from 15-20 weeks gestation. Results are shown below (mean ± SD). Mann-Whitney U test was used.

AFP:	Normal (n-78)	Abnormal (n=24)	Significance
Gestation (wks)	17.5 ± 1.7	18 ± 1.7	N.S.
PF4 (IU/ml)	1.3 ± 2.3	4.6 ± 10	p<0.001
BTG (IU/ml)	18.2 ± 13.8	30 ± 26.5	p<0.001

PF4 and BTG correlated significantly with AFP (expressed as MOM) only in the abnormal group (p<C.05, p<0.0001 respectively). We conclude 1) amniotic fluid PF4 and BTG are measurable in second trimester amniotic fluid, 2) are elevated in malformations where structural defects allow direct access to amniotic fluid or diffusion across simple membranes, and 3) may reflect underlying fetal pathology which activates platelets.

261 **FREQUENCY OF CONFINED PLACENTAL MOSAICISM IN PREGNANCIES WITH INTRAUTERINE GROWTH RETARDATION.** L. Wilkins-Haug, M.F. Greene, D.J. Roberts[X], C.C. Morton[X], Depts of Ob/Gyn and Pathology, Brigham and Women's Hospital, Harvard Medical School

Confined placental mosaicism (CPM) occurs in conjunction with a karyotypically normal fetus in 2-3% of chorionic villus samples. An increased rate of intrauterine growth retardation (IUGR) has been reported in these pregnancies. Among third trimester pregnancies complicated by IUGR, however, the frequency and clinical characteristics of CPM are unknown. We report karyotype analyses of amnion, chorion, villi and cord blood from 12 pregnancies with IUGR (birth weight <10%). In two cases, mosaicism was detected in all placental lines: 46,XX/48,XX,+17,+21 (55%/45%) and 46,XX/92,XXXX (66%/34%). The aneuploid line was confirmed in uncultured amnion preparations utilizing fluorescent in situ hybridization with an alpha satellite probe specific to chromosome 17. In a third case, a 46,XX/92,XXXX (75%/25%) mosaicism was confined to the amnion. In all cases, cord blood karyotypes were normal. Comparison of the ultrasound findings, perinatal complications and placental pathology from these cases provides a preliminary clinical description of the IUGR fetus with placental mosaicism. We conclude that CPM occurs with greater frequency among pregnancies with IUGR. Attempts to identify additional cases and to delineate further the associated clinical characteristics are ongoing.

262 THE EFFECTS OF ETHANOL ON LINOLEIC ACID INCORPORATION AND METABOLISM BY HUMAN PLACENTAL TISSUE IN VITRO. E. Kirk,[X] P. Ogburn, R. Holman,[X] J. Miles,[X] Dept. OB/GYN & Internal Medicine, Mayo Clinic & Hormel Institute, Rochester & Austin, MN

The teratogenic effects of ethanol (ETOH) may involve alterations in essential fatty acid metabolism. To study this, [1-^{14}C] linoleic acid (LA) was added to human placental tissue in DMEM media (n=7) exposed to 95% oxygen at 37° in a shaking incubator with varying concentrations of alcohol from 0-200 mg/dL. Samples of tissue and media were removed and analyzed at intervals over 24 hrs. We found significant uptake of LA into placental tissue (47.8% + 4.8% p<.01) at 12 hrs. ETOH did not significantly affect this uptake. After 12 hrs. incubation the conversion of LA to intermediate fatty acids leading to arachidonic acid decreased by more than 40% at even the lowest ETOH concentrations. This decrease was significant (p<.05) at ETOH concentrations of 100 & 200 mg/dL. Our work supports evidence of deranged LA metabolism secondary to ETOH in placental tissue in vitro.

263 **FREE BETA HCG IN DOWN SYNDROME SCREENING.** J. Larsen, K. Garver,[X] S. Frank,[X] J. Macri,[X] Dept. OB/GYN, George Washington Univ. Med. Ctr., Washington, D.C., Dept. Medical Genetics, Western Pennsylvania Hosp., Pittsburgh, PA, NTD Laboratories, Inc., Carle Place, NY.

Recent reports have demonstrated enhanced detection efficiency in Down syndrome screening using Free Beta hCG, especially in early weeks of gestation (<17 weeks). We have collaborated in a blind study using samples collected at a single center and maintained at -20°C. AFP and Free Beta were measured in a single laboratory utilizing in-house ELISA technology. Patient-specific risks were calculated using linear multivariate discriminant analysis. The normative data set for both MSAFP and Free Beta was established on 2,900 pregnancy samples, all of which were: under 35 years of age, between gestational weeks 14-22, non-diabetic, singleton, white pregnancies. The blind study set consisted of 50 patient samples, 42 of which were normal outcomes and 8 confirmed cases of trisomy 21. The blind study set consisted of 12 patients at or above 35 and 38 patients under 35 years of age. Nine cases fell below 17 weeks of gestation and 41 at or above. Of the 8 cases of Down syndrome, 6 (75%) demonstrated significantly increased risk and were thus correctly identified. In 4 cases where pregnancy outcome was normal, biochemical results indicated an increased risk for Down syndrome. In 3 of these cases, maternal age was greater than 35. We conclude that this is further confirmation of the usefulness of Free Beta hCG as a marker in Down syndrome screening.

264 EMBRYOSCOPIC OBSERVATIONS OF THE YOLK SAC

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Abnormalities of the secondary yolk sac have been associated with fetal malformations and death. To evaluate the appearance of the secondary yolk sac in normal and abnormal pregnancies, transcervical embryoscopy was performed on 220 pregnant women prior to termination of pregnancy. Gestational ages ranged from 7-13 menstrual weeks. The embryoscopic technique has previously been reported. Transvaginal ultrasound was performed prior to the termination. There were 8 fetal anomalies diagnosed by ultrasound that were included in the study, 4 of which had an aneuploidy. The where 4 additional aneuploid fetuses without an anomaly that underwent examination. The normal appearance of the secondary yolk sac in the first trimester is that of a highly vascular sphere, attached to the vitelline vessels. There were 3 yolk sac abnormalities noted in this study. An enlarged poorly vascular yolk sac was seen in a hydropic fetus at 9 weeks with an unbalanced translocation. In 2 cases where the fetus appeared normal a bilobed yolk sac was seen. **Conclusion**: Yolk sac abnormalities occur in normal and abnormal conceptuses and are not associated with most fetal anomalies.

265 THE USE OF NUCHAL SKIN FOLD MEASUREMENT IN SCREENING FOR CHROMOSOME ABNORMALITIES IN A HIGH RISK POPULATION

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The nuchal skin fold measurement is abnormal in more than 50% of fetuses with Down syndrome, but is not well investigated in other chromosome abnormalities. We prospectively measured the nuchal skin fold prior to amniocentesis in 800 fetuses at risk for chromosome abnormalities. The measurment could not be obtained because of fetal position or maternal obesity in 6.9%. Nuchal thickness was increased (>5mm) in 2.4% of normal fetuses. Thirty two abnormal karyotypes were identified, 7 of which were balanced familial translocations or inversions. A sonographic abnormality, nuchal thickness (NT) and/or dysmorphology (D), was found in 48% of the significant abnormal karyotypes. The positive predictive value of isolated fetal nuchal skin thickness for a significant abnormal karyotype was 12%.

Karyotype	N	D	NT	D+NT
Trisomy 21	9	1	3	2
Trisomy 18	3	2	0	1
Sex	7	0	0	0
Structural	5	2	0	0
Triploidy	1	0	0	1
Familial	7	0	0	0

Conclusion: High resolution ultrasound may identify nearly 50% of fetuses with significant chromosome abnormalities. Nuchal skin measurement does not appear to help identify fetuses with abnormal karyotypes other than Down syndrome.

266 HOW MUCH IS TOO MUCH NUCHAL MEMBRANE IN THE FIRST TRIMESTER?

Anthony Johnson, Patti Morgan[X], Shauna Heeger[X], Kimberly A. Klush[X], Ronald J. Wapner, Jefferson Medical College, Philadelphia, PA.

Given the strong association between aneuploidy and cystic hygroma in the first trimester we sought to determine if a similar relationship exists between fetal karyotype and the depth of the nuchal membrane thickness (NMT). In a prospective series of 827 consecutive viable singleton pregnancies with a risk of fetal aneuploidy ≥ a maternal age of 35 years, an attempt was made to obtain the NMT prior to chorionic villous sampling (CVS) performed at 9-12 weeks gestation. NMT was measured transabdominally with the fetus in the sagittal plane using the maximum distance from the outer cervical spine to the inner margin of the skin. Fetal position permitted NMT measurements in 242 cases (29.2%). Cytogenetic results were available in all cases, with 24 (2.9%) abnormalities identified. In normal fetuses with NMT measurements there were 230 normal results and 12 (5%) aneuploids. Excluding NMT findings, all fetuses included in the analysis were felt to be anatomically normal at the time of CVS. Linear regression failed to demonstrate a clinically significant difference in NMT range between 9 and 12 weeks in the normal fetuses, $r^2 = 0.032$.

NMT (mm)	≥2.0	≥2.5	≥3.0
sensitivity	81.8%	81.8%	63.6%
specificity	78.3%	90.4%	97%
+PPV	15.3%	29%	50%
-PPV	98.9%	98.9%	98.2%

Logistic regression confirmed a strong relation between NMT and fetal aneuploidy such that for each 1mm increase in NMT the relative risk of an affected fetus was increased by 4.67 (95% CI: 2.29-9.48, p<0.0001) We conclude that the presence of fetal NMT ≥ 2.0 mm in a first trimester pregnancy with a risk of fetal aneuploidy ≥ a maternal age of 35 years is highly predictive of a cytogenetic abnormality and warrants genetic counseling and possible prenatal diagnosis.

267 PRENATAL ALCOHOL EXPOSURE REDUCES AMNIOTIC FLUID LEVELS OF α-FETOPROTEIN IN RATS.

J. Hannigan[X], C. Flood[X], J. DiCerbo[X], G. Mizejewski[X]. Fetal Alcohol Research Center, Dept. of Ob/Gyn, Wayne State University School of Medicine, Detroit, MI and Wadsworth Laboratories, New York State Department of Health, Albany, NY.

Disruption of humoral neuro-regulatory factors is one possible mechanism of alcohol teratogenesis. In a study of pregnant alcoholics, Halmesmaki, et al (1987) predicted fetal alcohol syndrome (FAS) in 59% of cases, using maternal serum α-fetoprotein (AFP) as a marker. We tested the impact of prenatal alcohol on amniotic fluid AFP levels in rats. Alcohol (12.5 g/kg/day) was fed to dams from gestation day 6 to 20, when amniotic fluid was collected. Alcohol concentrations on this day were 120 mg/dl in maternal blood and 159 mg/dl in amniotic fluid. The alcohol-exposed litters had significantly lower amniotic fluid AFP levels than control groups. Alcohol-exposed fetuses weighed less and had larger placentae than control litters. Within the alcohol-exposed litters, there was a small negative correlation between amniotic fluid AFP and alcohol levels. Finally, alcohol-induced reductions in amniotic fluid levels of AFP were greater in male than in female fetuses. The results suggest that prenatal alcohol exposure reduces amniotic fluid AFP levels in a dose-dependent manner. Decreased availability of α-fetoprotein may contribute to alcohol-induced alterations in fetal maturation. SUPPORTED IN PART BY RSD AWARD No. 00111 FROM NIAAA.

268 **FETAL INTRACARDIAC KCL WITH SECOND TRIMESTER PREGNANCY TERMINATION: A METHOD TO AVOID THE HOPELESS RESUSCITATION OF THE NONVIABLE, ABNORMAL ABORTUS.** NB Isada, JC Fletcher, MP Johnson, WB Blessed, MI Evans. Division of Reproductive Genetics, Dept Ob/Gyn, Hutzel Hosp/WSU, Detroit, Mi and The Center for Biomedical Ethics, Dept Medicine, U Virginia, Charlottesville, Va.

Genetic and obstetric ultrasound services often reveal anomalous fetuses which, after counseling, parents choose to terminate. However, an increasing number of centers will not perform pregnancy terminations after 20 wks, in part because of the chance of obtaining a "live born" neonate. In a few cases, 2nd trimester abortion has resulted in the birth of a neonate with signs of life, even with utilization of intraamniotic urea. Pediatricians are put in an impossible position and may feel obligated to attempt full resuscitative efforts. Because this has happened in our institution, we have introduced fetal intracardiac potassium chloride (KCl) as a routine adjunctive procedure based on our experience with this method in multifetal pregnancy reductions. Under ultrasound guidance, a 22-gauge needle is directed into the fetal cardiac chambers. Proper placement is verified by blood return. Three to five cc of KCl (2 meq/ml) is instilled. Cessation of cardiac motion is verified with M-mode scanning. This approach was successful in causing rapid fetal cardiac arrest in 14 out of 15 cases. A decision to use intracardiac KCl avoids some of the ethical and legal quagmires that arise when the abnormal abortus-newborn has signs of life, and can be readily introduced into programs where invasive perinatal procedures are performed.

269 **NEUROTOXIC EFFECTS OF LEAD ON HYPOTHALAMIC DOPAMINERGIC NEURONS.** S. Ramin, W. Kedzierski,[X] J. Porter,[X] Dept. Ob/Gyn, Univ. Texas Southwestern Med. Ctr., Dallas, [X]

We investigated the neurotoxic effects of lead in primary cultures of hypothalamic cells from 18 to 22 day gestation Long-Evans rat fetuses. Two week old cell cultures were treated with various levels of lead nitrate for 24 h. Then, the medium was collected, acidified, and assayed for dopamine (DA) and dihydroxyphenylalanine (DOPA) by HPLC with electro-chemical detection. DOPA secretion is summarized below:

Lead	DOPA (pmol/well/24 h)		
Conc.	18 Days	20 Days	22 Days
Control	17.2±1.5	32.4±1.9	36.2±2.7
$1x10^{-10}M$	8.5±0.4	15.7±1.0	30.7±0.9
$1x10^{-9}M$	9.3±0.3	25.3±1.5	31.8±0.6
$1x10^{-8}M$	10.6±0.6	22.6±0.8	30.2±1.0
$1x10^{-7}M$	10.0±0.3	22.7±0.6	36.8±3.0
$1x10^{-6}M$	10.7±0.4	24.8±1.2	36.5±2.2
$1x10^{-5}M$	10.1±1.0	28.8±0.7	38.9±0.6
$1x10^{-4}M$	19.3±0.7	39.1±1.7	50.1±1.1
$1x10^{-3}M$	22.8±0.4	42.9±1.0	54.4±2.4

n=3-5

DA secretion follows the same pattern. At low levels of lead, DOPA and DA secretion are inhibited, whereas at high levels DOPA and DA secretion are stimulated. However, the inhibition with lead is more pronounced on cells from younger fetuses than it is from older fetuses. Conversely, the stimulation with lead is more pronounced on cells from older fetuses than it is from younger fetuses. The same pattern is seen with long term (two weeks) exposure of cultured cells to lead nitrate. These data suggest that the neurotoxic effects of lead may depend in part on the age of the fetus at the time of exposure.

270 **METHAMPHETAMINE USE DURING PREGNANCY IN A LARGE URBAN POPULATION.** S.M. Ramin, M.D., B.B. Little, Ph.D.,[-] K.J. Trimmer, M.D., D.I. Standard, B.S.,[+] C.A. Blakely, Ph.D.,[+] & L.M. Snell, M.P.H.[+] Depts. of Ob/Gyn & Fam. Prac. & Comm. Med., The Univ. of Texas Southwestern Med. Ctr., Dallas, Tx., PPRL, Texas A & M, College Station, Tx.

A paucity of information exists on effects of methamphetamine (MA) use during pregnancy and fetal outcome. Umbilical cord blood was collected from 863 consecutive births at two large urban public hospitals serving a primarily indigent population. Radioimmunoassay was used to test for the presence of MA. Medical record information was linked to serological analyses. Patients positive for other drugs (opiates, cocaine, alcohol, toluene) were excluded. Results are summarized below.

	MA (n=48)		Control (n=519)		
	Mean	SE	Mean	SE	P
Birth weight (gm)	3173	69	3327	18	0.03
Birth length (cm)	49.4	0.4	49.3	0.1	NS
Head circumference (cm)	33.7	0.3	33.8	0.1	NS
Apgar: 1 minute	8.4	0.1	8.6	0.1	NS
5 minute	8.8	0.1	8.9	0.1	NS
EGA (weeks)	38.6	0.3	38.8	0.1	NS
	N	%	N	%	
Major anomalies	2	4	27	4	NS
Minor anomalies	1	2	42	6	NS

These results are similar to those previously published for MA use during pregnancy by self-reported history. Although a reduction in birth weight was found in the MA group, the frequency of congenital anomalies was not increased compared to the control group.

271 **APPLICATION OF MOLECULAR CYTOGENETICS TO UNCULTURED FIRST TRIMESTER CHORIONIC VILLI.** K. Blakemore, G. Prabhakar,* G. Stetten,* R. Giraldez,* F. Marcus,* W. Chen.* The Johns Hopkins University School of Medicine, Baltimore, MD.

The clinical utility of fluorescent chromosome-specific centromere probes has been shown on interphase amniocytes and blood cells. We describe a method for use of these DNA probes on interphase nuclei from a solid tissue, chorionic villi. "Direct" preparations of uncultured villus cells were made by the method of Simoni et al. (1983), with and without 24 hr incubation, Colcemid exposure, and heat drying of slides. Fluorescence in situ hybridization was performed by the method of Pinkel et al. (1986) with minor modification. 20 ng of biotinylated probe DYZ3, DXZ1 or D9Z1 (ONCOR, Inc.) was added to a hybridization mixture (60% formamide/2X SSC), 10 μl of which was applied to each slide followed by heat denaturation (80°C for 5 min). Slides were incubated overnight at 42°C, and washed (60% formamide/2X SSC at room temp). The probe was detected with FITC conjugated avidin. Preparations were counterstained with propidium iodide (0.4 μg/ml) and examined using a Zeiss epifluoresce microscope. A clearer signal was obtained when slides were air dried. Omitting the 24 hr incubation and Colcemid exposure of the villi did not alter signal quality. Three cases illustrate the utility of this method: Case 1, at risk for the fragile X syndrome, to rapidly determine fetal sex; Case 2, a blighted ovum in a patient with two previous conceptuses, to demonstrate yet another recurrence; and Case 3, in which mosaicism for trisomy 9 was found on routine cytogenetic analysis of cultured villi, to examine the proportion of abnormal cells in direct villus metephases and interphase nuclei. Fluorescence in situ hybridization is a valuable new tool for rapid identification of fetal sex chromosomes and specific trisomies in prenatal diagnosis. First trimester results are possible using cell preparations made from chorionic villus tissue.

272 **ELEVATED MSAFP, PLACENTAL ABNORMALITIES, AND PRETERM DELIVERY.** **MA Williams,[X] DE Hickok, R Zingheim,[X] DA Luthy, J Kimmelman,[X] DA Nyberg,[X] BS Mahony,[X] Swedish Hosp. Med. Ctr. and University of Washington, Seattle WA**

Unexplained elevated MSAFP in the midtrimester is associated with placental abnormalities and adverse pregnancy outcomes. We assessed the association between unexplained elevated MSAFP and placental abnormalities in relation to infant outcomes in a hospital-based cohort study. Women with elevated MSAFP (N=188) had 4 times the risk of delivering a preterm infant than women with normal MSAFP (N=202)(95% CI 2.1-8.0).

GROUPS	N	% Preterm	RR (95%CI)
Normal MSAFP, Normal placenta	184	4.9	1.0
Elevated MSAFP only	149	17.5	3.6 (1.7-7.4)
Elevated MSAFP, Abnormal placenta	33	33.3	6.8 (3.1-15.2)

Women with both elevated MSAFP and abnormal placental findings were 6.8 times more likely to deliver a preterm infant than women with normal MSAFP levels and sonograms (95%CI 3.1-15.2). Elevated MSAFP was associated with a shortening of mean gestation length by 1.4 weeks (95%CI 0.6-2.0). A joint history of elevated MSAFP and placental abnormalities was associated with a greater decrease in the mean gestation by 2.8 weeks (95%CI 1.8-3.9). These findings suggest that elevated MSAFP and placental abnormalities are associated with particularly poor outcome. Careful examination for placental abnormalities should be a part of the evaluation of elevated MSAFP.

273 **ALPHAFETOPROTEIN IN DIABETIC PREGNANCY-A REASSESSMENT.** Tessie Tharakan*,Laxmi Baxi,Douglas Kramer*,Rosamond Anderson*,College of Physicians & Surgeons,Columbia University & Columbia Presbyterian Medical Center,New York,NY

We correlated glycosylated hemoglobin (HbA1) with Maternal Serum Alphafetoprotein (MSAFP) corrected for gestational age,race and maternal weight in 90, and amniotic fluid AFP (AFAFP) in 30, diabetic pregnancies.

Patient Groups	Time of HbA1 meas.	n	Corr.Coef. HbA1:MSAFP	P
1	in first 12 wks	22	0.06	ns
2	within 0-6 wks. before MSAFP	42	0.11	ns
3	within 6 wks after MSAFP	34	0.18	ns

There was no reduction in MSAFP with increasing HbA1 values. Even in patients with HbA1 exceeding 9 gm% (n=9) mean MSAFP was 0.84MOM(SD± 0.29) as compared to 0.85MOM(SD±0.23) in those with HbA1 from 5 to 8.9 gm%. It has been hypothesised that poor glycemic control may lead to decreased production of AFP by the yolk sac and /or the fetal liver. If so we would expect decreased AFAFP. We measured AFAFP in 30 pregnancies with HbA1 from 4.5 to 16% early in pregnancy, and under 7.5% (except 2 patients) at the time of amniocentesis. AFAFP values even in patients with HbA1 ⩾9%(n=3) were within normal limits. Thus,given these limited data, it appears that degree of control of blood sugar had no influence on fetal AFP production as indicated by AFAFP levels. If the levels of MSAFP in patients with poor glycemic control are low,this is probably not due to decreased fetal AFP production, but may perhaps be due to increased glycosylation of this protein causing increased catabolism. In conclusion,in this series of pregnancies with fairly well controlled diabetes,we observed normal values of AFAFP and MSAFP(mean 0.84 MOM) even when HbA1 exceeded 9%.

274 KARYOTYPE ANOMALIES IN FETUSES WITH URINARY TRACT ANOMALIES. S Sutherland*, J Iams, B Goodwin*, J Moore*, J Westman*, M Motley*, E Diss*, M Landon, R O'Shaughnessy, R Reiss, and S Gabbe. The Ohio State University Department of Obstetrics & Gynecology, and The Departments of Pediatrics and Cytogenetics Laboratory, Children's Hospital, Columbus, OH

The prevalence of abnormal karyotypes among fetuses with ultrasonographically detected urinary tract abnormalities is reported to range from 1 to 28%. The data available to guide clinical care is remarkably limited, however. We reviewed the clinical and ultrasound findings from 60 pregnancies in which a fetal urinary tract anomaly was detected antenatally. Among 33 in whom pre- or postnatal karyotype analysis was performed, there were five (15%) fetuses with abnormal karyotypes, two of whom had no extrarenal anomalies seen with ultrasound. Ten of 16 with uni- or bilateral cystic kidneys had a karyotype performed, and two had an abnormal karyotype: A fetus with trisomy 18 had oligohydramnios, a unilateral cystic kidney, and IUGR, and died *in utero*. One with 46, XX del (11)(q24.2) had absent amniotic fluid, bilateral renal cysts, and was aborted. Eight others with unilateral (6) or bilateral (2) cystic kidneys had normal karyotypes. Eight cases of megacystis were studied, and three of seven (43%) fetuses tested had abnormal karyotypes: one with a 46, XY/47,XXY karyotype and absent amniotic fluid was electively aborted; another with a cystic hygroma, megacystis, and normal fluid had an unbalanced translocation {46 XY, -12, +der(12) t(4;12)(q28;p13.3)}; a third with a balanced translocation [46,X,t(X:22)(q26;q11.1)mat] and oligohydramnios died in the neonatal period of pulmonary hypoplasia. Eleven of 19 fetuses (9 of 13 bilateral and 2 of 6 unilateral) with hydronephrosis had a karyotype done; all were normal. Eight of 17 with renal agenesis or dysplasia had studies done, and all were normal. Women presenting with a fetal renal anomaly should be offered antenatal chromosome studies.

275 SECOND TRIMESTER MSAFP IN INSULIN DEPENDENT DIABETICS. Sunderji S, M.D., Macri JN, Ph.D.[X] SUNY-Health Science Center at Syracuse, NY, NTD Labs at Carle Place, NY.

Attention has been drawn to the differing concentrations of maternal serum alpha-fetoprotein (MSAFP) in patients with insulin-dependent diabetes mellitus as compared to patients without this disease. We have evaluated this question in 132 insulin-dependent diabetic pregnancies. Our study confirms earlier findings that MSAFP concentrations in pregnant diabetic women are lower than non-diabetic women. However, our study fails to confirm earlier estimates of a 40% reduction. Our data indicates that the MSAFP concentration of pregnant insulin-dependent diabetics results in a 16% reduction compared to that observed in non-diabetic pregnant women.

It has been suggested that there is an inverse correlation between MSAFP and the degree of diabetic control during pregnancy as monitored by glycosylated hemoglobin. This has led some to recommend adjustment in MSAFP concentration in women whose diabetes has not been well controlled. Conversely, no correction should be made if diabetic control is satisfactory.

We studied the above association using glycated serum proteins which assesses average blood glucose levels over a 2-3 week period. The same maternal serum specimens from 132 insulin-dependent diabetic pregnancies that were assayed for MSAFP were also assayed for glycated serum proteins. A non-significant correlation of R = .10 was found (P = 0.27) for AFP vs. GSP. Our data suggest that the degree of control in maternal diabetes does not significantly influence or alter the level of MSAFP. The alteration in MSAFP observed in pregnant diabetic women may result from the underlying disease process rather than the level of blood glucose control.

We conclude that MSAFP concentrations in insulin-dependent patients should be increased by less than 20% rather than earlier recommended adjustments amounting to increase of as much as 66%.

276 **ANTENATAL DIAGNOSIS OF CONGENITAL FINNISH NEPROSIS:** A Ghidini*, M Alvarez, RL Berkowitz, G Silverberg*, E Ainbender*, CJ Lockwood. Mt Sinai School of Medicine, New York, NY and North Shore University Hospital, NY.

Congenital Finnish Nephrosis (CFN) is an autosomal recessive disorder requiring neonatal renal transplant for survival. The definitive diagnosis of CFN rests on electron microscopic evaluation of the epithelial foot processes and basal membrane of the glomeruli. The prenatal diagnosis can be suspected in the presence of a positive family history with an amniotic fluid (AF) alpha-fetoprotein (AFP) > 10 Standard Deviations (SD) accompanied by a negative AF acetylcholin-esterase (AchE), absent hemoglobin F (HbF) and unremarkable fetal sonographic examination (1). We have expanded this indications to a low-risk population where the diagnosis of CFN has been correctly suspected in 7 patients with a MSAFP > 2.5 Multiples of the Median based on an AF AFP > 10 SD associated with a normal ultrasound examination, negative AchE and absent HbF (see Table below). In case # 6 the diagnosis of CFN was further supported by the presence of an AF albumin concentration of 960 mg/dl (normal values < 400 mg/dl).

Case#	wks	AFAFP (SD)	AchE	HbF	Fetal Renal Pathology*
1	22	>100	neg	neg	CFN
2	18	90	neg	neg	CFN
3	19	11	neg	neg	CFN
4	18	10	neg	neg	CFN
5	17	15	neg	neg	CFN
6	20	113	neg	neg	CFN

* = confirmed by election microscopy

CONCLUSIONS: The diagnosis of CFN can be strongly suspected even in a low risk population by the presence of an AF AFP > 10 SD. An elevated AF albumin concentration may represent an additional marker for the diagnosis of CFN; however its precise significance awaits further study.

(1) Ryyanen M, et al: Br J Obstet Gynaecol 1983; 90:437-42.

277 **ENHANCED DOWN SYNDROME SCREENING EFFICACY BY COMBINING MATERNAL SERUM AND FETAL BIOMETRIC MARKERS.** *S Rotmensch, M Liberati,* JS Luo,* T O'Connor,* MJ Mahoney,* JA Copel, JC Hobbins, A Baumgarten.* Depts OB/GYN, Genetics, and Lab Med, Yale University.*

Whether fetal sonographic biometry and maternal serum alphafetoprotein (MSAFP) are independent parameters, which in combination could enhance screening efficacy for Down syndrome (DS), has not been determined. We analyzed prospectively collected data on 1125 patients who had genetic amniocentesis for advanced maternal age between 15-21 gestational weeks. 27 DS fetuses were identified in this population. Regression equations relating BPD to humeral length (HL) and femoral length (FL) were used to calculate ratios of observed (O) to expected (E) length. A cutoff point of 0.88 O/E for HL and FL yielded sensitivities (Sn) of 25.9% and 14.8%, and specificities (Sp) of 95.1% and 95.1%, respectively. A MSAFP cutoff of 0.42 MoM yielded a 37.0% Sn, and 98.1% Sp. Logistic regression analysis confirmed that O/E ratios for HL and FL were independent of MSAFP as a predictor of DS. A combination of O/E for HL and MSAFP yielded 55.5% Sn and 93.8% Sp. Inclusion of both HL and FL O/E ratios did not improve sensitivity. Conclusions: MSAFP and fetal long bone biometry are independent predictors of DS fetuses, which in combination enhance screening efficacy.

278 **DOES SONOGRAPHIC DETERMINATION OF FETAL SEX ENHANCE SCREENING EFFICACY FOR DOWN SYNDROME?** *S. Rotmensch, M Liberati,* JS Luo,* T O'Connor,* MJ Mahoney,* JC Hobbins, A Baumgarten*, Depts OB/GYN, Genetics, and Lab Med, Yale Univ.*

Male fetuses produce 5-10% higher maternal serum alphafeto-protein concentrations (MSAFP-C) than female fetuses. Postnatal extremity length is also larger in males than in females. We examined midtrimester sonographic biometry in relation to fetal sex and analyzed it's utility for enhancement of DS screening efficacy in conjunction with sex-related MSAFP-C. The series included 565 male and 558 female fetuses (including 14 males and 13 females with Down syndrome [DS]) whose mothers had amniocentesis for advanced maternal age between 15-21 weeks gestation. Regression equations predicting humeral length (HL) from biparietal diameter (BPD) for males and females were different (P=0.0006). A 0.84 ratio for females and 0.88 ratio for males of observed (O) to expected (E) HL yielded sensitivities (Sn) of 15.4% and 30.7% and specificities (Sp) of 98.0% and 95.1%, respectively. Median MSAFP for male fetuses was 2% higher than in females. An MSAFP of 0.5 MoM for females and 0.52 MoM for males yielded a Sn of 30.8% and 71.4% and Sp of 96.4% and 93.6%, respectively. Logistic regression analysis confirmed independence of O/E HL and MSAFP as predictors of DS. Combination of these parameters yielded 46.1% Sn and 94.3% Sp for female DS fetuses, but no improvement of the 71.4% sensitivity for male DS fetuses. Sex specific DS screening would result in overall sensitivity of 58.3% (assuming 90% feasibility of sonographic sex assignment and 92% prediction accuracy), as compared to 55.5% without knowledge of sex. **Conclusions:** 1. Male and female fetuses display differential biometric features in midtrimester. 2. Fetal sex specific interpretation of DS screening results tends to improve sensitivity. This would be more apparent with a 5-10% difference in MSAFP-C, as reported from large series in the literature.

279 **PEPTIDE HETEROGENEITY OF HUMAN CHORIONIC GONADOTROPIN (hCG) AND ITS ß-SUBUNIT IN DOWN SYNDROME PREGNANCIES.** S Rotmensch, M Liberati,* A Kardana,* M Mahoney,* JC Hobbins, LA Cole. Depts OB/GYN and Genetics, Yale University

Maternal serum hCG and its free ß-subunit (ß-SU) are markers for midtrimester Down syndrome (DS) screening. hCG molecules, however, are heterogeneous and have discordant immunoreactivity which can correlate with missing peptide linkages ("nicking") in the ß-SU. The purpose of this study was: 1. to compare hCG and ß-SU peptide heterogeneity between normal and DS pregnancies; 2. to determine the effect of nicking on hCG and ß-SU screening assays for DS. Sera from 60 women with karyotypically normal (51) and DS (9) fetuses at 13-22 weeks gestation were examined by immunoenzymometric assays utilizing monoclonal antibodies specific for total (=intact and nicked) hCG and total ß-SU as well as intact hCG and intact free ß-SU. **Results:** The amount of intact hCG (as % of total hCG) was 83.4%± 5.8% in DS and 76.9±11.1 % (p=0.06) in karyotypically normal pregnancies. Accordingly, the percent of nicked hCG was not statistically different. However, the assay specific for total free ß-SU was significantly higher in DS (0.34 ±0.18 vs.0.24±0.08%, p=0.04). The assay specific for intact free ß-SU showed no difference between DS and normal pregnancies (0.15±0.09 vs. 0.16±0.09 % p=NS). Accordingly, Down syndrome pregnancies had a higher serum concentration of nicked free ß-SU (0.20±0.18% vs. 0.03 ±0.08%, p=0.04%). In individual Down syndrome cases 8 fold higher serum concentrations of ß-SU were detected by the total ß-SU assay, as compared to the ntact ß-SU assay. **Conclusions:** 1. Missing peptide linkages in the ß-sububit occur more frequently in maternal sera of DS pregnancies. 2.Immunoassays which do not recognize nicked ß-SU yield falsely lowered concentrations and should not be used for DS screening. 3.Whether differences in nicking can be utilized to enhance DS screening efficacy remains to be determined.

280 **COCAINE USE DURING PREGNANCY IN A LARGE URBAN POPULATION.** Bertis B. Little, M.A., Ph.D.,+ Kenneth J. Trimmer, M.D., Susan M. Ramin, M.D., Donna I. Standard, B.S.,+ Craig A. Blakely, Ph.D.,+ and Laura M. Snell, M.P.H.+ Depts. of Ob/Gyn and Family Practice and Community Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas, Public Policy Research Laboratory, Texas A & M University College Station, Texas.

Umbilical cord blood collected at delivery from 97% of 890 consecutive births at two large public county hospitals was tested for a cocaine metabolite (benzoylecgonine) by radioimmunoassay (RIA). Medical record information was linked to serological analyses. Any patient positive for other drugs (methamphetamines, opiates, alcohol, toluene) was excluded. Infants whose cord blood was positive for the cocaine metabolite (n=126) had significantly (P<.01) reduced birth weight (by >200 grams), birth length (1.5 cm), and head circumference (1 cm) compared to seronegative controls. Frequencies of genitourinary and major congenital anomalies were significantly (P<.05) increased among drug-exposed infants (odds ratios = 3.4 and 2.4, respectively) compared to seronegative controls. None of the mothers gave a history of cocaine use during pregnancy but birth outcomes parallel those reported previously in this population based upon self-reported history or drug use. Screening for cocaine abuse should be considered when unexplained complications such as fetal growth retardation or congenital anomalies are clinically suspected.

281 RAPID CYTOGENETIC ASSESSMENT OF FETAL BLOOD SAMPLES. Richard P. Porreco, M.D., Beryl Harshbarger, CLSp(CG)*, Loris McGavran, Ph.D.*, Presbyterian/St. Luke's Medical Center, The Children's Hospital, Denver, Colorado 80203.

Abnormalities diagnosed by antenatal ultrasound frequently require cytogenetic analysis of the fetus. Occasionally the karyotype results may alter obstetric management. Eighteen patients had fetal blood sampling done for a variety of abnormalities diagnosed by antenatal assessment. These included hydrocephalus, oligohydramnios, intrauterine growth retardation, non-immune hydrops, duodenal atresia, polyhydramnios, and multiple anomalies. Four cytogenetic techniques were attempted in arriving at a fetal karyotype. These included a direct harvest of lymphocytes in the fetal blood sample, a 24 hour incubation of fetal lymphocytes without mitogen, and a 48 and 72 hour mitogen stimulated incubation followed by harvest and analysis. Six of the eighteen cases showed diagnostic cytogenetic abnormalities. One half of the cases had results reported within 30 hours of obtaining the specimen following analysis of unstimulated cultures, including three of the six abnormal results.

#	Gest.Age	Indication	Results	Time (da)
1	38 wks	Hydrocephalus	46,XY	2
2	20 wks	Hydrocephalus	47,XX,+21	2
3	36 wks	IUGR, anomalies	69,XXX	<1
4	24 wks	Oligohydramnios	46,XY	2
5	18 wks	Down syndrome in affected twin	47,XY,+21	2
6	24 wks	Fetal ascites, oligohydramnios	46 XY	<1
7	30 wks	Non-immune hydrops	No Growth	-
8	17 wks	Cystic hygromas, hydrops in twin	No Growth	-
9	35 wks	IUGR	47,XY,+21	<1
10	31 wks	IUGR	46,XX	<1
11	19 wks	Identical twins, hydrothorax	46,XX	3
12	18 wks	Non-immune hydrops	46,XY	1
13	26 wks	IUGR	46,XY	2
14	26 wks	Oligohydramnios	46,XX	<1
15	26 wks	Hydrocephalus	46,XX	1
16	36 wks	Multiple anomalies	47,XY,+18	2
17	25 wks	Duodenal atresia	46,XY,-13, +robt(13;21)	1
18	30 wks	Hydramnios	46,XX	1

Conclusion: Unstimulated lymphocyte cultures from fetal blood samples may provide rapid cytogenetic analysis and alter obstetric management.

282 BART'S HYDROPS FETALIS IS NOT UNIFORMLY FATAL. D. Jackson, R. Farmer, K. Murray, D. Bianchi, J. Akabutu, Dept. M-F Medicine and Pediatrics, U of Ca, Irvine, Harvard Med. School, U of Alberta, Canada.

Fetal non-immune hydrops secondary to homozygous alpha thalassemia (4 gene deletion, Bart's hydrops fetalis) is generally counseled as a uniformly fatal perinatal condition. Follow-up on three known survivors refute this counseling. The children are now ages 8, 7, and 3. All received aggressive neonatal resuscitation at birth including intubation and blood transfusions. Gestational age and mode of delivery were 32 weeks/C-section, 28 weeks/C-section, and 28 weeks/vaginal-breech delivery, respectively. Neonatal and infant characteristics are shown below.

Current Age	Hgb Electrophoresis (Bart\Portland\H)	NICU (Day's)	Ht. / Wt. Percentile		Motor/Neuro Devel.
8 y/o	80%\20%\0	48	10%	5%	Bilat.hearing aids/poor speech dev.
7 y/o	78%\19%\3%	73	10%	25%	Appropriate for age
3 y/o	93%\7%\0	64	10%	5%	Appropriate for age

All three children undergo monthly transfusion and chelation therapy.

Conclusions: The follow-up on three neonatal survivors with in utero Bart's hydrops fetalis refutes counseling of uniform mortality. For parents opting for prenatal diagnostics and pregnancy continuation, in utero transfusion/gene therapy may be a focus of future perinatal research efforts to augment neonatal management.

283 SERUM HUMAN CHORIONIC GONADOTROPIN (hCG) AS A MARKER FOR LOW BIRTHWEIGHT IN WOMEN WITH UNEXPLAINED ELEVATIONS IN MATERNAL SERUM ALPHA-FETOPROTEIN (MSAFP). Hurley TJ, Quirk, Jr. JG, Blacklaw M*, Walker G*, Miller C* and O'Brien TJ*. University of Arkansas for Medical Sciences, Little Rock, AR, Department of Ob/Gyn.

It has been previously reported that unexplained elevations in MSAFP obtained in the second trimester of pregnancy are associated with an increased incidence of adverse pregnancy outcomes including stillbirth and low birthweight (LBW). In an attempt to more precisely identify this high risk group, we evaluated the efficacy of serum hCG measurements in 82 women with MSAFP elevations (>2.5 multiples of the median), but without demonstrable fetal defects by level II ultrasound or amniocentesis. In these 82 women, the incidence of (LBW) (<2500g) (including fetal demise) was 23% (19) vs 5% (5) in a control group of 100 women with normal MSAFP's (p<.001). For the study we defined an abnormal hCG as (≥2.5 MoM or ≤0.5 MoM). The incidence of an abnormal hCG value in these 82 women was 24%. In this latter group 50% delivered infants of birthweight <2500 grams as compared to 14.5% of those with serum hCG within the normal range (p<.002). There was one still birth in each group. The test performed with a sensitivity of 52%, specificity of 85% and a positive predictive value of 50%. Therefore women with an abnormal hCG (≥2.5 MoM or ≤0.5 MoM) and an elevated MSAFP have a 1:2 chance of delivering a low birthweight infant as opposed to a 1:5 chance with an elevated MSAFP alone. Further work needs to be done on the utility of serum hCG and other biochemical markers as independent predictors of poor obstetrical outcome in women with unexplained elevated MSAFP's.

284 **FETAL CHOROID PLEXUS CYSTS: AN INDEPENDENT RISK FACTOR FOR CHROMOSOMAL ANOMALIES.**

Porto M, Murata Y, Warneke LA[X], Keegan, Jr KA.
University of California Irvine Medical Center, Orange CA.

This prospective, controlled study was designed to determine the inherent risk of fetal aneuploidy with sonographically detected choroid plexus cysts (CPC) in the 2nd trimester. 63 cases of CPC were detected in 3247 2nd trimester ultrasound examinations (1.9%). Our control group consisted of all patients who had an ultrasound examination and genetic amniocentesis, between 15 and 22 weeks gestation, by the same sonologist on the same day as a study subject with a CPC (N=211). The two groups were demographically similar in maternal age and indication for examination (primarily: maternal age, abnormal AFP, previous anomaly). Six chromosomally abnormal fetuses: Trisomy 18 (3), Down syndrome (2), and Klinefelter syndrome were found in the CPC group (6/63, 9.5%). One fetus with Trisomy 21 was encountered in the control group, (1/211, 0.5%) [$p < 0.002$]. There was no correlation between bilaterality and chromosomal anomalies. Of note, 5 of 21 (23.8%) fetuses with CPC greater than 5mm in diameter had aneuploidy, compared with only 1 of 42 (2.4%) chromosomal anomalies with smaller cysts ($p < 0.02$). Two fetuses with CPC and aneuploidy had no other sonographically detected anomalies despite targeted scans including echocardiography. We conclude that fetal CPC is an independent risk factor for chromosomal anomalies. Based on our data, all patients with CPC should receive genetic counseling and be offered prenatal karyotype analysis.

285 EARLY AMNIOCENTESIS - RELIABILITY AND SAFETY. A LONGITUDINAL FOLLOW-UP TO DELIVERY IN 400 CONSECUTIVE CASES. S. Iwanicki,[x] M. Pattinson[x], D. Cox[x], H. Pattinson[x], D. King[x], Alberta Hereditary Diseases Program, Calgary, AB, Canada

Since 1989, advanced maternal age (≥35 y at EDC) patients in our center have been given the option of early amniocentesis (EA, 9-14 weeks). Patients were counselled that local risk rates related to EA were unknown. The procedures followed a detailed ultrasound (US) assessment and the taps were performed under US guidance by an experienced obstetrician. Cell cultures were processed for chromosome preparations using the flask method when EA was performed ≤13 weeks, otherwise the in situ method was used. Results were monitored to determine the incidence of culture failure (CF). Patients were contacted 4 weeks post EA to assess for early complications and again 4-6 weeks post-partum to evaluate for late complications. There were no stillbirths or neonatal deaths. Results are summarized in the table below with the percentage for each gestational age.

Gestational age (wks)	9	10	11	12	13	14	TOTAL
No. of procedures	3	46	73	83	74	121	400
Culture failure	1(33)	6(13)	1(1.4)	0 (0)	1(1.4)	0 (0)	9(2.3)
Pseudomosaicism	0 (0)	0 (0)	0 (0)	0 (0)	4(5.4)	1(0.8)	5(1.3)
Leakage/Bleeding	0 (0)	2 (4)	2(2.7)	0 (0)	2(2.7)	1(0.8)	7(1.8)
Spontaneous Abortion	0 (0)	1(2.2)	0 (0)	2(2.4)	1(1.4)	2(1.7)	6(1.5)
Pregnancy termination	1(33)	0 (0)	0 (0)	0 (0)	2(2.7)	0 (0)	3(0.8)
Preterm delivery	0 (0)	0 (0)	1(1.3)	1(1.2)	2(2.7)	3*(2.5)	7(1.8)
Congenital anomalies	0 (0)	1(2.2)	1(1.3)	1(1.2)	2(2.7)	1(0.8)	6(1.5)
Lost to Follow-up	1(33)	1(2.2)	0 (0)	1(1.2)	1(1.4)	1(0.8)	5(1.3)

* Includes one following abdominal trauma and one twin pregnancy

Early experience showed that CF at <11 weeks was unacceptably frequent (14.3%). Procedures were then performed only at ≥11 weeks, with a CF rate of 0.6%. There were no more congenital abnormalities (2 club foot, 1 absent patellae, 1 cleft lip, 1 cleft palate and 1 congenital hip dysplasia) than would be expected. This study suggests that the risks and reliability associated with EA compare favourably to those previously reported with traditional amniocentesis.

286 OUTCOME OF ANTENATALLY DIAGNOSED CYSTIC ADENOMATOID MALFORMATIONS (CAM)

Jeffrey Kuller, Jerome Yankowitz[X], James Goldberg, Michael Harrison[X], Roy Filly[X], Peter Callen[X], Mitchell Golbus[X] Fetal Treatment Program Univ. Calif. Med. Ctr., San Fran., CA

We reviewed our experience with 21 cases of fetal CAM diagnosed antenatally. Seventeen of these patients elected to continue pregnancy. In 9 cases, non-immune hydrops fetalis (NIHF) did not develop and all these infants survived. In the remaining 8 cases, NIHF developed between 20-27 weeks. Fetal intervention was undertaken in 7 of the 8 cases. In the 1 case with NIHF in which intervention was not undertaken, the patient ruptured membranes at 33 menstrual weeks. The infant died of respiratory distress at 1 hour of life. In 3 cases, ultrasound guided needle aspiration of macrocystic lesions was performed. In one patient, preterm labor and delivery of a viable infant occurred 1 week later. In the other 2 fetuses, cystic fluid rapidly reaccumulated and cystoamniotic shunts were placed. In one patient, the catheter remained in place but the patient ruptured membranes 2 days later and delivered a previable fetus. In the other case, the catheter malfunctioned 2 days later and the patient underwent fetal surgery. In 5 patients, fetal surgery was performed (hysterotomy/resection of chest mass). Three infants survived and are doing well. One case is ongoing. The nonsurvivor died on day 2 of life with anasarca and severe respiratory compromise. The mother's course was complicated by severe preeclampsia ("mirror syndrome"). In cases where NIHF occurs early, fetal surgery may prove to be a viable therapeutic option.

287 **MANAGEMENT OF FETAL HEMOLYTIC DISEASE NOT REQUIRING ANTENATAL TRANSFUSION THERAPY.** C. Weiner, L. Estle[X], K. Wenstrom and S. Sipes. Dept. OB/GYN, Univ. Ia. College of Med., Iowa City, Ia. 52242

Direct evaluation of a fetal blood rather than an amniotic fluid specimen permits the accurate identification of the fetus at risk to develop antenatal anemia secondary to maternal red cell alloimmunization *(AmJObGyn, Oct 1991)*. Based on the recommended nomogram, 60% of affected fetuses require ≤2 cordocenteses and are permitted to deliver at term. Of the remaining group, 80% require transfusion therapy. We sought the ***risk of unexpected postnatal anemia and/or hyperbilirubinemia in those fetuses who prospectively were felt not to be at risk for antenatal anemia.*** Follow-up is presently available on 35/40 (88%) completed at risk pregnancies. 71% were delivered by their referring physician at 38 ± 2w (range 34-41 w) with a BW of 3140 ± 565 gm. 24/35 (69%) had been placed in low to moderate risk groups and 69% underwent ≤2 cordocenteses. No procedure was performed after 35 w. The HCT at delivery was 46 ± 9% (range 24-58%) in the 17 neonates tested. There was one anemic neonate at birth (2.8%). On review, it was discovered that the risk pattern assigned to this fetus was in error. A second cordocentesis 4 weeks after the first should have been performed at 34w when this fetus may have already been anemic. Five (14%) underwent 1 or more double volume exchange transfusions for hyperbilirubinemia, and 4 (11%) received a simple transfusion for anemia that developed ≥ 48hrs after birth. 23/35 (66%) required some phototherapy (23-240 h). **CONCLUSION:** ***1) The development of fetal anemia can be accurately predicted weeks prior to delivery. 2) Affected but nonanemic neonates remain at risk for hyperbilirubinemia and hemolytic anemia and should be delivered in at least a secondary level hospital.***

288 TWIN TO TWIN TRANSFUSION. J. Castaner,[X] C. Cetrulo, M. D'Alton; Dept. of MFM, St. Margaret's Hospital for Women/Tufts Univ. School of Medicine, Boston, MA

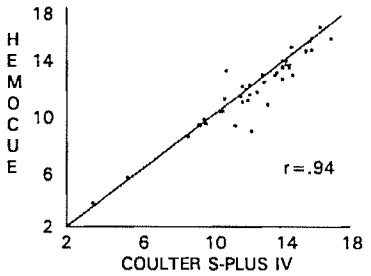
Eighteen cases of twin to twin transfusion sydrome (TTS) were revieved to elucidate if gestational age at presentation or obstetrical management affected perinatal outcome. All cases met 2 or more criteria: 1) like sex twins with monochorionic placentas (18 pts.), 2) weight differences >20% (13 of 18), 3) polyhydramnios/oligohydramnios (17 pts.), 4) "stuck twin" (7 pts.), 5) hydrops fetalis (3 pts.), or 6) difference in hemoglobin >5 gm. (6 pts.) Obstetrical management consisted of conservative observation in 12 cases, decompression amniocentesis in 4 cases and Indomethacin therapy in 2 others. Eight cases presented before 25 weeks and 10 cases after 26 weeks. The perinatal mortality (PNM) rate was 12.5% for those with conservative management, 25% for the amniocentesis group and 75% for those treated with Indomethacin. In the amniocentesis group reversal of hydrops was visualized in 1 case. All the perinatal deaths were confined to the group who presented before 25 weeks gestation. The PNM rate was 22.2% for the total group, 50% for those presenting <25 weeks and zero if presentation was after 26 weeks. Three patients with one IUFD continued their pregnancy for another 4, 10, and 14 weeks respectively. One case developed IUGR, microcephaly, multicystic encephalomalacia. The other 2 pregnancies proceeded to 34 and 35 weeks respectively with uneventful outcomes. In conclusion, we observed that TTS presenting before 25 weeks gestation demonstrates poor outcome irrespective of management while those presenting after 26 weeks has a zero PNM rate. Because of the high PNM associated with Indomethacin we discourage its use. Decompression amniocentesis needs further study to elucidate its role in management of TTS.

289 RENAL FUNCTION AFTER IN-UTERO INTERVENTION FOR FETAL OBSTRUCTIVE UROPATHY. Melissa Fries,[X] Mary Norton,[X] James Goldberg, Michael Harrison,[X] Roy Filly,[X] Peter Callen,[X] Ruth Goldstein,[X] Mitchell Golbus[X]. Univ. CA, San Francisco

In-utero vesicoamniotic shunting and vesicostomy by open fetal surgery have been used to relieve fetal urinary outflow obstruction, theoretically preventing pulmonary hypoplasia and preserving renal function. Past studies have suggested that hypotonic fetal urine (Na $\leq$100meq/dl; Cl $\leq$90meq/dl; osmolality $\leq$210mOsm/l) predicted fetal survival with normal renal function. Twenty-four cases of fetal intervention (16 shunts; 8 open surgeries) were performed from 1981-1991, with shunts producing 7 survivors, 4 neonatal demises (NND), and 5 TAB's; and surgery leading to 3 survivors, 4 NND, 1 TAB. Five of the 7 shunt survivors had hypotonic urine electrolytes and ultrasonically-normal kidneys; values were hypertonic on 1 survivor and not studied on the other. All 7 had variable renal dysfunction at birth, with Cr 1.1-9.0 mg/dl and BUN 18-56 mg/dl. Renal transplant is planned for 3 pts. Of shunt non-survivors (9 pts), only 2 had hypotonic urine. In the fetal surgery survivors, 1 had hypotonic urine and one had borderline hypertonic values (osm=221); both these pts. have normal renal function. The other survivor had hypertonic urine (osm=255) and has had renal transplant. Of the non-survivors, electrolytes were not studied in 2 pts. and were hypotonic or borderline hypertonic (osm=215) in the remaining 3. NND were from complications of prematurity. Hypotonic urine may predict survival but not good long term renal function for fetuses treated by intervention. A randomized trial of intervention vs non-intervention and early delivery appears indicated.

290 FETAL HEMOGLOBIN QUANTITATIONS USING THE HEMOCUE® SYSTEM ARE FAST AND ACCURATE. S.M. Berry, M. Dombrowski, W.B. Blessed,[X] J.A. Bichalski,[X] T.B. Jones, D.B. Cotton, Dept. of Ob/Gyn, Hutzel Hosp./WSU, Detroit, MI

Rapid and accurate quantitations of fetal hemoglobin (hgb) levels during cordocentesis (PUBS) are critical in making the decision to perform an intrauterine transfusion, and in deciding how much blood to transfuse. Hemocue (weight = 700g) provides Hgb levels in 45 seconds. Accuracy of the Hemocue system has not been adequately validated for prenatally obtained fetal blood. We compared fetal Hgb's from the Hemocue versus those from the Coulter S-Plus IV on 44 fetal specimens obtained via PUBS. Gestational age (GA) = 26.7 ± 5.7 wks (range = 18 to 37).



The Hemocue values ranged from 3.3 to 16.4 gm/dl. GA and extremes in Hgb levels were not significantly correlated to Hemocue values by stepwise regression. Conclusions: 1) The Hemocue system is fast and accurate for fetal Hgb quantitations throughout gestation, despite varying Hgb F/ Hgb A_1 ratios. 2) The accuracy of the Hemocue system is not significantly affected by extremes in fetal Hgb levels.

291 FETAL PLATELET COUNTS IN RED CELL ALLOIMMUNIZATION CORRELATE WITH THE SEVERITY OF THE DISEASE. George R. Saade, M.D.,[X] Kenneth J. Moise, Jr., M.D., Michael A. Belfort, M.D.,[X] Diane Hesketh, R.N.,[X] Robert J. Carpenter, Jr., M.D.; Dept. of Ob/Gyn; Baylor College of Medicine; Houston, Tx.

Thrombocytopenia has been noted in hydropic neonates with HDN and its presence could complicate intrauterine vascular transfusions (IVT). **Purpose:** To determine whether any subgroup of fetuses with red cell alloimmunization have lower platelet counts (Plt). **Material and Methods:** The records of 53 patients undergoing IVT for red cell alloimmunization were reviewed. The pre-transfusion fetal Plt was compared between hydropic and non-hydropic fetuses and with the fetal bilirubin, hct, and retic count at the initial IUT. In addition, the hct was adjusted for gestational age by calculating the number of standard deviations (#SD) below the mean for that age. The student t test and the Pearson R coefficent were used to analyze the data. **Results:** 29 fetuses were hydropic; 24 non-hydropic. Hydropic fetuses had a significantly lower Plt count than non-hydropic fetuses (195.8 ± 96.0 vs 250.0 ± 78.6 $10^3/mm^3$; p = .02), with 31% and 17% of hydropic fetuses having plt counts less than 150,000 and 100,000/mm^3 respectively. Plt counts correlated positively with the hct (r = .65; p <.01) and negatively with the retic count (r = .65; p <.01), and the hct #SD below the mean (r = .65; p <.01), but did not correlate with bilirubin (r = .07; p = NS). **Conclusions:** Hydropic and severely anemic fetuses are at risk for thrombocytopenia. We suggest that increased erythropoeisis drives the hematopoietic stem cell away from platelet production.

292 TRANSVAGINAL EMBRYONIC ASPIRATION (TEA) - A SAFER METHOD FOR SELECTIVE REDUCTION IN MULTIFETAL GESTATION

Itskovitz J[X], Drugan A[X], Levron J[X], Thaler I[X], Brandes JM[X]. Dept. Ob/Gyn, Rambam Medical Ctr. and Faculty of Medicine, Technion, Haifa, Israel

Selective reduction of fetal numbers in high multifetal gestation is usually performed transabdominally at 10-12 wks gestation with a pregnancy loss rate of 15-25%. We performed TEA in 18 multifetal pregnancies at 7-8 wks of gestation. In 17 cases, the initial fetal number of 3-7 was reduced to 2; in 1 case, the number of embryos was reduced from 4 to 3. None of the remaining fetuses vanished following the procedure. In 17 cases, the procedure was accomplished in 1 session, with 1 or 2 needle insertions. 1 patient required 2 sessions for reduction from quintuplets to twins. Mean procedure time was 10 minutes. 15 patients delivered successfully at/after 35 wks and 2 patients at 32 wks. The procedure-related loss rate was 5.6% - 1 premature delivery at 25 wks. We suggest that TEA is safer than the transabdominal route and simpler to perform; and the earlier procedure may be more acceptable to patients from moral and religious points of view.

293 **IN-UTERO FETAL ELECTROCARDIOGRAM: A METHOD FOR EVALUATING FETAL ARRYTHMIAS**

Mark T Cullen MD, Jacqueline J Green RDMS, Luis Sanchez-Ramos MD. University of Florida, Jacksonville, FL.

Fetal tachyarrhythmias can be associated with fetal decompensation, hydrops and death. While the in-utero medical treatment of fetal cardiac arrhytamias is promising, proper drug selection is dependent on an accurate diagnosis of the dysrhythmia. The incorrect choice of medication could lead to a worsening of the condition or fetal death. Diagnostic tests available include real time ultrasonography and M mode echocardiography. M mode echocardiography can be difficult to perform and interpret, requiring significant expertise and sophisticated equipment. The purpose of this study is to describe a new method and equipment for direct in-utero evaluation of the fetal heart rate. The technique utilizes a fetal electrode that is placed transabdominally through a 20g spinal needle performed similar to a transabdominal amniocentesis. Under ultrasound guidance, the thin wire electrode is threaded through the needle and comes in contact with the fetal skin. The electrode is grounded to the maternal abdomen and attached to a fetal monitor (Advanced Medical Systems, Inc. model IM76, Hamden, CT). A fetal electrocardiogram is printed to the monitor strip or transferred to an electrocardiograph. The technique has proven effective when tested on fetuses prior to termination of pregnancy.

294 **OUTCOME IN REPEAT PREGNANCIES AMONG BLACK AND WHITE TEENAGERS.**

SP Cliver*, RL Goldenberg, CA Hickey,* J Jin,* ML Blankson.* University of Alabama Hospitals, Birmingham, Alabama.

Teenage women are at higher risk than adult women for delivering growth retarded or preterm infants. To assess these risks in repeat teen pregnancies, first and second pregnancies of 737 indigent teenagers who delivered singletons were compared. The population studied was 80% black, 20% white, and 30% had a first delivery before age 15. Fetal growth retardation (FGR) fell from 9.3% in the first pregnancy to 4.2% in the second ($p=.0001$), and the preterm delivery (PTD) rate (<37 weeks gestation) rose from 14.8% to 18.9% ($p=.04$). The PTD rate in white teenagers fell slightly while the rate in black teens rose from 15% to 21% ($p=.02$). Maternal height and self-reported use of cigarettes, alcohol, and street drugs did not change significantly from the first to the second pregnancy. Maternal body mass index (BMI), the proportion of married women, and gestational age at first prenatal visit all increased significantly from the first to the second pregnancy ($p<.05$). The mean number of prenatal visits decreased by 2 visits ($p=.0001$). In a multivariate analysis adjusting for race, gestational age, infant sex, age at first delivery, and interval between pregnancies, the increase in mean birthweight in the second pregnancy was partly explained by an increase in maternal BMI from the first to the second pregnancy. For teenagers whose first pregnancy resulted in PTD, the recurrence rate was 46%, compared to a PTD rate of 14% for teenagers whose first delivery was at term ($p<.001$). The recurrence rate was especially high for black teens (49%) compared to white teens (32%) despite a significantly greater weight gain between pregnancies in blacks ($p=.02$) as well as a longer interval between pregnancies ($p=.004$). Black teens, however, presented two weeks later for care in both pregnancies and had fewer prenatal visits. Previous PTD in teenagers appears to confer a greater risk for recurrence than in adults, particularly among black women.

295 **THE NATURAL INTERLEUKIN-1 RECEPTOR ANTAGONIST PREVENTS INTERLEUKIN-1-INDUCED PRETERM PARTURITION.**

R. Romero, M. Mazor, B. Tartakovsky[X], Depts. of Ob/Gyn, Yale Univ. School of Med., New Haven, CT; Wayne State Univ., Detroit, MI; Soroka Med. Center, Ben Gurion Univ., Israel; The Weizmann Institute, Rehovot, Israel

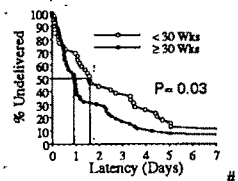
Interleukin-1 (IL-1) has been implicated in the mechanism responsible for preterm labor in the setting of infection. The participation of IL-1 in this process has important diagnostic, prognostic and therapeutic implications. Indeed, patients with IL-1α and IL-1β in the amniotic fluid have a high rate of intraamniotic infection and frequently fail to respond to tocolysis. Recently, a new member of the IL-1 gene family, the natural IL-1 receptor antagonist (IRAP), has been isolated, purified and cloned (Nature 1990;343:341 and 1990;344:633). This polypeptide blocks prostaglandin biosynthesis in several cell types and thus may have a role in the treatment of preterm labor. The purpose of this study was to determine if IRAP can prevent IL-1-induced parturition in mice (AJOG 1991;165 [in press]). Materials and Methods: A clinical trial was conducted in 17-day-pregnant mice (BALB/C) impregnated by B6D2 F-1 males. Mice were randomly allocated into four groups: group 1, placebo (saline); group 2, IL-1; group 3, IRAP (Upjohn, Kalamazoo, MI); group 4, IL-1 and IRAP. All compounds were administered in one subcutaneous injection. Results: 1) IRAP prevented IL-1-induced parturition and other behavioral IL-1-induced biological effects; 2) IL-1 induced preterm labor and delivery in all cases within 18 hours post-injection; 3) IRAP administration was not associated with any demonstrable side effects including vaginal bleeding. Conclusion: IRAP prevented IL-1-induced preterm labor and delivery. These data suggest that anti-cytokine agents may have value in the treatment of preterm labor associated with infection and open new therapeutic horizons for preterm parturition.

296 MAGNESIUM SULFATE IS A POOR INHIBITOR OF OXYTOCIN-INDUCED UTERINE CONTRACTILITY IN PREGNANT SHEEP. Margaret L. Watt-Morse, Steve N. Caritis, Jye Ping Chiao[X], University of Pittsburgh, Magee-Womens Hospital, Pittsburgh, PA

We have previously demonstrated that magnesium is a poor inhibitor of oxytocin-induced contractility in sheep. Steady state concentrations were maintained for only 2-3h in that study, but clinical studies suggest that magnesium sulfate requires more time to inhibit uterine contractility. In the present study, we evaluated inhibition of oxytocin-induced uterine contractility by magnesium sulfate after 24h of infusion. We inserted catheters in the femoral artery and vein and amniotic cavity of 6 pregnant sheep between 110 and 117 days gestation (term = 147). Two to four days after surgery, animals received a loading dose of magnesium sulfate followed by maintenance infusion to achieve magnesium concentrations of 6.1-7.8 mEq/L. Animals received 500 mU boluses of oxytocin prior to and after 4 and 24h of infusion. Uterine activity was quantified by integrating the area under the time uterine pressure curve. Mean inhibition of uterine contractility was 18% after 4h and 24h of magnesium infusion. Previous work from our laboratory shows that uterine contractility induced by the same oxytocin dose are inhibited 48-64% when ritodrine concentrations are 1-11 ng/ml. When compared with ritodrine, magnesium sulfate is a poor inhibitor of oxytocin-induced myometrial contractility in pregnant sheep.

297 OUTCOME OF MULTIPLE GESTATION COMPLICATED BY PRETERM PROM (pPROM). B Mercer[X], L Crocker[X], M Dahmus[X], F Pierce[X], B Sibai. University of Tennessee, Memphis.

Currently there is little information as to the latency period and perinatal outcomes after pPROM in multiple gestations. Specifically, the relative risks of morbidity and mortality between the presenting and non-presenting infants have not been addressed. The purpose of this clinical investigation is to report the natural history and clinical outcome in 101 pregnancies having this complication. **Method:** The study population included all such pregnancies managed at this institution over a 10 year period. Outcome variables studied included: latency from membrane rupture to delivery, maternal infectious morbidity including placental histology, and neonatal morbidity with special emphasis on differences between the presenting (A) and non-presenting (B) fetus. **Results:** The mean gestation at pPROM was 30.0±4.4 weeks (range 18-36). There were 2 triplet gestations. Eight were delivered on admission because of advanced gestation. The median latency of the 93 remaining pregnancies was only 1.08 days, with 91% delivering within 7 days. The latency to delivery, stratified by gestational age at rupture, is summarized in the Figure (analysis by Life-table methodology). Pathologic chorio-amnionitis was identified in 29% of placentas (22/75), and 29.7% developed clinical chorioamnionitis. Outcomes of the 204 infants are summarized in the Table. Three infants > 600 grams were stillborn. **Conclusions:** The multiple gestation complicated by pPROM is associated with a brief latency regardless of gestational age. Paired analysis reveals no difference in infant survival, but a significant increase in respiratory morbidity in the non-presenting infant.



	A	B
Birth weight (gr.)	1468	1463
% 5 min Apgar <7	17.4	28.7*
% HMDs	7.9	22.8*
% Resp. insufficiency	21.4	18.5
% Intubation > 24 hrs	16.0	24.8**
% O_2 Rx > 24 hrs	23.4	29.6*
% Sepsis	18.0	13.0
% Survival#	90.8	90.0

birthweight >600 gr. *P<0.05 ** P= 0.08

298 SAFETY AND EFFICACY OF THE OXYTOCIN ANTAGONIST ATOSIBAN IN THREATENED PRETERM LABOR: INITIAL U.S. TRIAL. TM Goodwin, RH Paul (Univ. of So. California, Women's Hospital, Los Angeles, CA), H Silver (UC Davis), M Parsons, R Chez, W Spellacy (Univ. of So. Florida), R Hayashi (Univ. of Michigan), L North,[X] R Merriman[X] (RWJ Pharmaceutical Research Institute).

Atosiban (deTVT/DETO-OXY) is a potent antagonist of oxytocin which lacks significant cardiovascular, pulmonary, and central nervous system activities. A double blind, placebo controlled trial was conducted to test the hypothesis that intravenous Atosiban (300 µg/min) is more effective than bedrest in reducing the frequency of preterm contractions. 120 women between 20 and 36 weeks gestation with >4 uterine contractions/hour were randomized to receive placebo (N=60) or Atosiban (N=60) for 2 hours. Eight subjects (4 Atosiban and 4 placebo) were excluded after randomization. The two groups did not differ in demographic characteristics or cervical findings. Baseline contraction frequency, as determined by external tocodynamometry was similar in Atosiban and controls (15.1±4.8 vs. 16.8±6.7, mean±SD). The overall mean decrease in contraction frequency was 8.2±5.8 for Atosiban compared to 4.6±5.8 for controls (p=0.001) In patients receiving Atosiban, 27/56 (48%) had ≤4 contractions/hour during the second hour of infusion compared to 11/56 (20%) of controls (p=0.003). Five Atosiban patients had cervical change compared to 8 controls (NS). Mild gastrointestinal symptoms were noted in 2 Atosiban patients and in one control. **Atosiban is more effective than bedrest in reducing preterm uterine activity. No significant adverse effects were observed with a two hour infusion. We are currently studying the safety and efficacy of Atosiban in patients with preterm labor evidenced by cervical change.**

299 AMNIOTIC FLUID LAMELLAR BODY COUNT: A RAPID AND RELIABLE FETAL LUNG MATURITY TEST. C. Dalence,[X] L. Bowie,[X] J. Dohnal,[X] E. Farrell,[X] M. Vye,[X] Evanston Hospital and Northwestern University Medical School, Evanston, IL

The lamellar body count (LBC), a rapid and quantitative test for fetal lung maturity (FLM), has previously been shown to correlate with other tests for FLM. Lamellar bodies are structures secreted by fetal Type II pneumocytes and consist almost entirely of surfactant phospholipids. In this study, we compared the ability of the LBC vs a lung phospholipid profile to predict the respiratory outcome of 118 well documented cases. Fetal lung phospholipid profiles were performed by thin-layer chromatography. Maturity was indicated by a ratio of phospholipids (lecithin+PG+PE+PI)/sphingomyelin ≥3:1. LBC was determined for all amniotic fluid specimens using the platelet channel of a conventional Coulter counter. Calibrated spheres and commercial controls were used to verify the accuracy of the particle counts. Data and predictive values (PV) are summarized below:

Clinical	Lung Profile		LBC		LBC	
Outcome	<3:1	≥3:1	≤30K	>30K	≤10K	>10K
RDS	10	1	14	0	10	4
No RDS	9	92	44	60	9	95
Sens/Spec	0.91/0.91		1.00/0.58		0.72/0.91	
+PV/-PV	0.53/0.99		0.24/1.00		0.53/0.96	

Using a value greater than 30,000/uL to predict FLM, the LBC showed no false negative results while the phospholipid profile had one false negative. Therefore, the LBC demonstrated 100% sensitivity in the critical function of predicting the absence of RDS. Furthermore, we found the number of false positives indicated by the LBC could be minimized by using a second cutoff of 10,000/uL to indicate a high-risk for RDS. LBC values between 10,000/uL and 30,000/uL appear to be of intermediate risk for developing RDS (4 of 39 cases). Conclusion: The LBC appears equivalent to traditional phospholipid determination in predicting fetal lung maturity. However, the LBC offers considerable advantage over traditional phospholipid determination based upon its simple technique and rapid availability.

300 NEONATAL MORBIDITY BETWEEN 34-37 WEEKS' GESTATION. M.D. Fox,[X] J.F. McCaul, R.W. Martin, W.E. Roberts, B. McLaughlin,[X] J.C. Morrison, Dept. Ob/Gyn, Univ. Mississippi Med. Ctr., Jackson, MS

Objective: To determine the risk of significant neonatal morbidity between 34-37 weeks in women with preterm labor (PTL) who are not given tocolytic therapy. Study Design: Women between 34 and 37 weeks' gestation with documented PTL and intact membranes were given informed consent and offered entry into this prospective trial. Population: One hundred and one women met inclusion/exclusion criteria; 90 gave informed consent and were randomized. Intervention: Women were randomized by a disinterested third party (pharmacy) from a random number table to receive either intravenous magnesium sulfate tocolysis followed by oral therapy using magnesium gluconate tocolysis (treatment group - T) or conservative management with hydration, sedation and observation (control group - C). Main Outcome Measured: The incidence of maternal side effects from tocolysis, interval from diagnosis of PTL to delivery, birth weight, and neonatal outcome were noted. Results: Of the 90 women entering the study, 45 in T and 45 in C, two discontinued tocolytic therapy because of gastrointestinal side effects. There were no serious neonatal complications. In both T and C there were 3 who had TNN and 1 RDS in each group.

	N	GA at diagnosis (weeks)	Interval to delivery (days)	Birth weight (grams)	GA at delivery
T	45	34.9 ± .7	15.7 ± 12.0	2741 ± 496	36.5 ± 1.7
C	45	35.1 ± .7	15.4 ± 13.6	2762 ± 585	37.7 ± 1.9

The gestational age on admission, cervical dilatation at PTL diagnosis, interval to delivery, and birth weight were not significantly different between T and C. Conclusion: Neonatal morbidity following delivery between 34 and 37 weeks' gestation is unchanged whether or not attempts to arrest labor are successful. The extra expense and maternal risk of tocolysis is not justified by beneficial results in the infant.

301 **PRETERM PREMATURE RUPTURED MEMBRANES IN THE TWIN GESTATION: A CASE CONTROL STUDY**

Montgomery DM[X], Perlow JH[X], Asrat T, Morgan MA, Bahado-Singh RO, Garite TJ Long Beach Memorial Womens Hospital, Long Beach, CA. University of California Irvine Med Center, Orange, California

The natural history of premature preterm ruptured membranes (PPROM) in the twin gestation has not previously been described. Therefore, we sought to describe the clinical course of PPROM in the twin gestation, and to determine if perinatal outcomes are similar in twin and singleton pregnancies complicated by PPROM. Over the previous decade, 80 sets of twins between 25-36 weeks estimated gestational age (EGA) presented with PPROM (TProm) to our institution. A control group (n=80) consisting of singletons matched for EGA at time of PPROM (SProm) was selected and perinatal outcome between groups was analyzed. Maternal demographic characteristics were not significantly different between groups. During the study period, our general management protocol for PPROM was expectant and did not include use of tocolysis, antibiotics, or steroids.

Outcome	Tprom(%)	Sprom(%)	P value
EGA at PPROM(Wk)	30.5 ± 4.5	30.3 ± 4.7	NS
Latency period			
<48 hr	70.0	65.0	NS
49hr-7da.	16.3	21.3	NS
>7da.	13.8	13.8	NS
Chorioamnionitis	18.8	23.6	NS
Fetal distress	0.05	0.14	NS
RDS	35.0	35.0	NS
Neonatal infection	18.8	21.3	NS
Neonatal death	11.3	13.8	NS

The natural history of PPROM in the twin gestation parallels that in the singleton pregnancy and suggests that similar antepartum management strategies are appropriate for both groups.

302 **A COMPARISON OF FIVE METHODS OF AMNIOTIC FLUID FETAL LUNG MATURITY TESTS: SENSITIVITY, SPECIFICITY, AND COST EFFECTIVENESS OF INDIVIDUAL VS. COMBINED TESTS.** **Chris T. Sloan, M.D.; Robert P. Lorenz, M.D.; Claire Michael, M.D.[X]; Division of Maternal Fetal Medicine and Department of Clinical Pathology, William Beaumont Hospital, Royal Oak MI and, Wayne State University**

OBJECTIVES: 1) To compare the sensitivity and specificity of commercially available rapid tests and chromatographic tests of fetal maturity and 2) propose an algorithm for cost effective utilization of tests. METHODS: A retrospective study of lecithin/sphingomyelin(L/S), phosphotidylglycerol(% PG)(Gluck method), FLM[R](Abbott TDx), Lumedex FSI[R](Beckman), and Amniostat[R](Hana), and newborn outcome was performed. Direct costs were estimated for materials and labor. RESULTS: 153 pregnancies (35.7 +/- 2.6 weeks gestation) were analyzed, respiratory distress syndrome occurred in 13 (8.5%). Diabetic pregnancies were analyzed separately. For nondiabetics (n=102), the number of studies, definition of maturity, sensitivity (for the outcome of RDS), and specificity for each test was: L/S n=80, 2.0, 92%, 88%; PG n=80, trace,75%, 79%; FLM n=91, >=60,100%, 85%; FSI n=67, >47, 80%, 66%; Amniostat n=39, ≥ 0.5, 67%, 55%. For diabetics (n=51): L/S n=48, 2.0, 100%, 94%; PG n=48, trace, 100%, 92%; FLM n=46, >=60, 100%, 87%; FSI n=41, >47, 100%, 68%; Amniostat n=23, ≥ 0.5,(sens=??(no RDS), 61%. Testing sequences were compared for sensitivity, specificity, and direct costs. CONCLUSIONS: This retrospective study suggests the least costly method is a first step using the FLM[R] (Abbott TDx), then an L/S and %PG only for the 26% that are immature by FLM. This method has a sensitivity of 90% , a specificity of 92%, and an average direct cost of $27.40 in our nondiabetics and should be prospectively studied.

303 THE EFFECT OF PHYSICIAN OFFICE VISITS ON UTERINE ACTIVITY AS DETERMINED BY HOME UTERINE MONITORING. T.L. Bennett, M.D. , P Winchester, M.D.[X] , B.E. Finley, M.D. , Humana Hospital of Overland Park, KS and University of Kansas Medical Center, Kansas City, KS

Life-style modifications, with limitation of both physical activity and mental-stress, are quite often an integral part of the management of the pregnant woman at risk for preterm delivery. In-office clinical evaluations necessitate that women at risk for preterm labor leave the home environment with a curtailment of prescribed rest. To determine the effect of physician office visits on uterine activity, home uterine monitoring records of 24 women at risk for preterm labor were retrospectively reviewed. The average activity was determined for the morning (AM) and the late afternoon (PM) for days with and without physician visits. No statistical difference was found between the AM uterine activity for days with and without physician visits. A statistically significant rise in uterine activity the PM after a physician office visit was seen when compared to the average PM activity for those days without such visits (paired t-test $p<0.02$). This increased activity tended to subside over the next 24 hours. In conclusion, uterine activity as recorded by home uterine monitoring, is increased subsequent to a prenatal office visit in those woman previously determined to be at risk for preterm delivery. The implications of this "white-coat labor" should be considered in protocols to evaluate and manage woman at risk for preterm events.

AVERAGE UTERINE ACTIVITY (Contractions in One Hour)

	No Physician Visit		Physician Visit	
	AM (n=24)	PM(n=24)	AM (n=24)	PM(n=21)
Mean	3.19	3.06	3.84	5.75
S D	1.53	1.96	2.54	3.74

304 **A RANDOMIZED COMPARATIVE TRIAL OF INDOMETHACIN AND SULINDAC FOR THE TREATMENT OF REFRACTORY PRETERM LABOR.** S.J. Carlan, W.F. O'Brien, T.D. O'Leary[X], D.S. Mastrogiannis[X]. Dept. Ob/Gyn, Univ. of South Florida College of Med. Tampa, FL

This study was designed to investigate the efficacy and safety of sulindac (clinoril) in the treatment of preterm labor. Thirty-six women in preterm labor who had failed initial attempts at tocolysis with magnesium sulfate were randomized to receive either oral indomethacin or oral sulindac for 48 hours. Mean gestational age at admission was 29 weeks and 30 weeks for the sulindac and indomethacin groups respectively. There was a significantly greater hourly fetal urine output, depth of deepest amniotic fluid pocket, and amniotic fluid index in the sulindac group. Fetal ductus arteriosus flow velocities were elevated in the indomethacin but not the sulindac group. The drugs had similar rates of success in delaying delivery for both 48 hours and 7 days. Mean birth weight was 2000 grams and 2323 grams for the sulindac and indomethacin group respectively. Sulindac appears to be as effective as indomethacin when used to treat refractory preterm labor, but with fewer fetal side effects.

(Mean ± SD)	Sulindac	Indomethacin	P
EFW at admission	1492 ± 384	1608 ± 453	NS
Cervical dilatation at admission	2.8 ± 1.7	2.8 ± 1.0	NS
Cervical dilatation after $MgSO_4$ failure	3.5 ± 1.5	3.6 ± 1.4	NS
Serum magnesium at randomization	5.9 ± .9	5.6 ± .9	NS
L/S	1.4 ± .5	1.4 ± .5	NS
Fetal urine production at 24 hrs. (cc)	15 ± 2	9 ± 2	<.001
Interval delivery 2 days	19 ± 24	24 ± 23	NS
Delivery delayed >48 hrs.(%)	88	83	NS
Delivery delayed >7 days (%)	55	61	NS
Umbilical artery pH	7.3 ± .07	7.3 ± .1	NS
Number of NICU days	25 ± 30	16 ± 26	NS

305 **A COMPARISON OF PREDISCHARGE SURVIVAL AND MORBIDITY IN SINGLETON AND TWIN VERY LOW BIRTHWEIGHT INFANTS.** E.J. Wolf, A. M. Vintzileos, T. Rosenkrantz[X], J.F. Rodis, L. Lettieri, A. Mallozzi[X], Univ. of Connecticut Health Center, Farmington, CT

The perinatal mortality rate of twins is 4 - 11 times higher than that of singletons and they are widely reported to have more morbidity than singletons, mainly due to higher preterm birth and anomaly rates. However it is not clear that liveborn preterm twins suffer greater morbidity than comparable singletons. In fact, twins have been reported to develop pulmonary maturity earlier than singletons and this might result in decreased morbidity relative to comparable preterm singletons. This retrospective review of 496 consecutive singleton and 104 twin infants weighing 500-1499 grams and born alive at 24 through 31 weeks gestational age was conducted to determine if predischarge survival and morbidity in VLBW twin infants were greater than that of comparable singletons. The mean (± SD) gestational age of the singletons was 27.5 (±2.0) weeks and for the twins was 27.6 (±2.0) weeks. There were no statistically significant differences in mean gestational age, gestational age distribution, mean birthweight, birthweight distribution, gender or race between the two groups. The predischarge survival rate for twins (77%) was not significantly less than that of singletons (82%). There were no statistically significant differences between twins and singletons in the incidence of neonatal respiratory distress syndrome (63 vs 71% respectively), pulmonary interstitial emphysema (14 vs 16%), patent ductus arteriosus (28 vs 29%), necrotizing enterocolitis (3 vs 5%), intraventricular hemorrhage (11 vs 16%), retinopathy of prematurity (11 vs 18%) or survival to discharge (23 vs 18%). The incidence of bronchopulmonary dysplasia was significantly less in twins (28%) vs. singletons (46%) (p=.001). These results suggest that very low birthweight twins do not necessarily suffer greater morbidity or mortality than singletons of comparable birthweight and gestational age and actually develop less bronchopulmonary dysplasia.

306 **DOES THE PREGNANCY COMPLICATION RESULTING IN PRETERM DELIVERY INFLUENCE PREDISCHARGE SURVIVAL AND MORBIDITY?** E. Wolf, A. Vintzileos, T. Rosenkrantz[X], J. Rodis, J. Mead, S. Gray[X], Univ. of CT Health Center, Farmington, CT.

This retrospective study was conducted to determine how the principal pregnancy complication leading to preterm delivery influences predischarge survival and morbidity of VLBW infants. The hospital records of 535 consecutive liveborn singleton infants weighing 500-1499 grams were reviewed and five primary complications resulting in preterm delivery were identified: 1) PROM (n=244 or 46%); 2) idiopathic preterm labor or PTL(n=97 or 18%); 3) Antepartum hemorrhage (n=58 or 11%); 4) PIH (n=98 or 18%); and 5) "other" indications (n=38 or 7%). Neonatal records were studied to determine the presence of RDS, bronchopulmonary dysplasia (BPD), pulmonary interstitial emphysema (PIE), PDA, NEC, IVH, retinopathy of prematurity (ROP) and infant death prior to hospital discharge. The mean (± SD) gestational age and birthweight were 27.9 (± 2.4) weeks and 1032 (± 263) grams, respectively. The EGA of infants delivered due to PIH (29.3 ± 2.0) and "other" indications (30.0 ± 3.1) was slightly greater than that of PROM (27.6 ± 2.2), PTL (26.8 ± 1.0) and hemorrhage (27.1 ± 1.9) infants ($p < .05$). There were no statistically significant differences in mean birthweight, race, and discharge survival rates (range 71-83%) between the 5 groups. Those delivered due to PROM developed significantly less RDS (63%), BPD (40%), and PDA (19%) than the PTL (77%, 57%, and 38%, respectively) or hemorrhage (81%, 59%, and 41%, respectively) groups ($p<.05$). The PTL group developed more PIE (26%) and IVH (31%) than the PROM (11% and 11%) or hemorrhage (9% and 13%, respectively) groups ($p<.05$). The PIH group developed more RDS (74%), PIE (20%) and PDA (35%) than the PROM group (62%, 11% and 19%, respectively) and developed less BPD (33%) and IVH (11%) than the PTL group (57% and 31%, respectively). In summary, the principal pregnancy complication resulting in preterm delivery significantly influenced predischarge morbidity but not predischarge survival of liveborns.

307 **TACHYPHYLAXIS TO RITODRINE IS DEPENDENT ON RITODRINE CONCENTRATION AND NOT ON MAGNITUDE OF BETA-ADRENERGIC RECEPTOR LOSS.** Ashi Daftary, M.D., Steve Caritis M.D., Jye Ping Chiao, M.S. University of Pittsburgh School of Medicine, Pittsburgh, PA

Infusion of ritodrine, for 24 hours, leads to a decrease in myometrial beta-adrenergic receptor (BAR) density. This is associated with a decrease in ritodrine's ability to maintain inhibition of oxytocin-induced myometrial contractions, i.e., tachyphylaxis. In order to determine if the magnitude of the receptor change is dose-dependent, we utilized an *in vivo* model to define the relationship between ritodrine concentration, myometrial BAR density, and loss of contraction inhibition. Ritodrine was infused continuously for 24 hrs in 27 chronically-catheterized sheep (GA 110-120 d; term=147d). Myometrial biopsies were obtained before and after the infusions and receptor density was quantified using standard techniques with tritiated dehydroalprenolol. Intermittent oxytocin boluses of 500 mu were given into the aorta and uterine response was measured. Infusion rates between 0.12 and 4.0 μg/kg/min yielded ritodrine concentrations from 3 to 156 ng/ml. No significant correlation was found between ritodrine concentration and the magnitude of decrease in BAR density ($R^2=0.137$, $p>0.05$); the average decrease in BAR density was 34%. Loss of BAR did not reduce ritodrine's ability to inhibit oxytocin-induced uterine contractions. Ritodrine did, however, lose it's effectiveness when plasma concentrations exceeded 40 ng/ml. Thus, tachyphylaxis to ritodrine is dependent on the ritodrine concentration and not the magnitude of BAR loss.

308 IMPROVING LONG TERM MENTAL AND NEUROLOGIC OUTCOMES IN INFANTS < 1500 G OVER PAST 5 YEARS. W.J. Morales, D. Hurford, S. Schorr, Orlando Regional Medical Center, Orlando, FL

Over 10 year period, 1981-1991, 1,605 infants < 1500 g were admitted to NICU and of these 809 were born in the second half of the decade. Mental and neurologic examinations were performed on 603 infants at corrected one year of life, 300 born from 1986-1990. The mean birth weight was 796 gm for the first 5 years as compared to 809. While no significant improvement was achieved in the last 5 years in neonatal survivor (88 vs 85%), RDS (59 vs 59%), ROP (27 vs 22%) and NEC (2 vs 2%), there has been a significant decrease in the incidence of BPD (23 vs 16%), IVH - total (43 vs 30%), IVH - grade 3, 4 (21 vs 12%) and more importantly, in the proportion considered to have NORMAL development - (MDI and PDI>80, no blindness or chronic lung disease or cerebral palsy) - 66% vs 79%. These encouraging findings remain valid when groups were compared at different birth weight groups < 750 g, 750-999 g, 1000-1249 g, 1250-1500 g. Improved long term neonatal outcomes were related to antenatal combined with neonatal pharmacology therapy in the form of combined steroids and TRH along with artificial surfactant in addition to advances in perinatal and neonatal outcome.

309 CLINICAL DECISION ANALYSIS IN PRETERM PREMATURE RUPTURE OF THE MEMBRANES. Bebbington, Michael W. Grace Hospital, Vancouver B.C. Canada.

Preterm premature rupture of the membranes (PPROM) is responsible for a significant amount of perinatal morbidity and mortality. There are no consistent guidelines that help to answer the question; At what gestational age should delivery take place when presented with PPROM? A non-recursive decision analysis model was developed to answer this question. Analysis was carried out for three separate gestational ages; 26, 30 and 34 weeks. Utility values were determined for each of the outcomes using a standard gamble technique. Probability values for each branch of the model were determined using values from data at our institution. As expected, at 26 weeks the model preferred the conservative therapy option, while at 34 weeks, the immediate delivery option was preferred. At 30 weeks, the preferred treatment option varied with how the patient ranked the outcomes of pregnancy prolongation and intact survival of the newborn. If preference was given to prolonging the pregnancy the model preferred conservative therapy whereas if intact survival was given priority then the immediate delivery option was preferred. This study shows the value of clinical decision analysis in perinatal medicine and the importance of patient input into management decisions.

310 THE EFFECT OF SUBCUTANEOUS TERBUTALINE INFUSION ON UTERINE ACTIVITY IN PATIENTS AT RISK FOR PRETERM DELIVERY. P.J. Weinbaum AND M. Olson, Dept. OB/GYN, Albany Medical College, Albany, N.Y. AND Healthdyne, Inc., Marietta, GA.

Continuous subcutaneous infusion of Terbutaline via pump (T-pump) has recently been utilized as tocolytic therapy for ambulatory management of preterm labor. The concomitant use of home uterine activity monitoring allowed us to examine the effect of T-pump therapy on the pattern of uterine activity. A group of 202 patients at risk for preterm delivery were monitored for a minimum of 10 days prior to the onset of preterm labor and tocolytic therapy. The mean gestational age (GA) at the initiation of monitoring was 28.5 wks. and 30.1 wks. at start of T-pump. Mean frequency of uterine contractions over that 10 day period was 3.3/hr., with an increase to 6.6/hr., over the final 72 hrs. This increase in uterine activity was associated with an increase in subjective symptoms and/or cervical change in the majority of patients. T-pump was initiated and over the first 24 hrs. mean uterine activity was significantly decreased to 3.3/hr. With appropriate alterations in either bolus or basal infusion dosage, contractions were maintained at a mean of 3.9/hr. over the duration of T-pump therapy lasting an average of 4.9 wks. Repeat hospitalization for excessive uterine activity unresponsive to home manipulation of T-pump occurred in only 9.6%. Mean GA at delivery was 36.2 wks. and 70% of patients completed 35 wks. This study confirms the observation of a significant increase in monitored uterine activity shortly before the onset of preterm labor. It also suggests that T-pump rapidly and effectively decreases mean uterine activity and sustains this effect delaying delivery for a clinically significant period.

311 EXPANDED PUBLIC FINANCING OF PRENATAL CARE: IMPACT ON BRONX PRETERM BIRTHS 1985- 1989. JE Deaver, Albert Einstein College of Medicine (AECOM), Dept Ob/Gyn, Bronx, New York.

There have been few rigorous birth outcomes evaluations of policies designed to reduce financial barriers to utilization of prenatal care. The New York State Prenatal Care Assistance Program (PCAP), from 1985-1989, provided prenatal care to women lacking health insurance with incomes between 100% (the Medicaid limit) and 185% of the poverty level. The present study hypothesizes that PCAP-eligible patients who were not enrolled in the program (i.e. self-pay) had increased rates of preterm birth compared to PCAP enrollees. Prenatal, birth outcome, and PCAP enrollment data in the POPRAS database of the AECOM were linked using Oracle and Structured Query Language (SQL) to produce 11,013 complete records. Logistic regression analysis with SPSS determined that self-pay status was a significant predictor of preterm birth (p=0.0354, odds 1.38) along with previous preterm birth (p=0.00005, odds 1.95) and delivery in the impoverished South Bronx (p=0.00005, odds 2.14) while age, race, ethnicity, gestational age at onset of care, location of prenatal care, and program year were not predictive. Selection bias is an important consideration with respect to interpretation of these data since the assignment to PCAP versus self-pay was not random. However, it appears that eligible patients who did not participate in a public prenatal care financing program experienced increased rates of preterm birth compared to participants even while all other factors were controlled.

312 **AMNIONIC FLUID INDEX AND PREGNANCY OUTCOME IN PATIENTS WITH PREMATURE RUPTURE OF THE MEMBRANES.** M.Hussey*, N.Carlson, R. Besinger, J.Gianopoulos, Loyola Univ. Med. Ctr., Maywood, IL.

Retrospective analysis of 127 singleton pregnancies with premature rupture of membranes (PROM) between 25 and 35 weeks gestation was performed. Patients included presented within 72 hours of rupture and had no evidence of labor, infection, bleeding or fetal distress. Patients who received tocolysis or corticosteroids were excluded. Patients were divided into three groups based on their four quadrant amniotic fluid index (AFI) at admisssion. Complications and outcome (severe variable decelerations (SVAR), amnionitis, latency period, 1 and 5 minute Apgar scorus less than 7) were compared. There was no difference in incidence of cesarean section (C/S), birth weight or perinatal mortality.

	GROUP 1	p (1,2)	GROUP 2	p (2,3)	GROUP 3	p (1,3)
AFI (CM)	< 4		≥4,<8		≥8	
N	59		52		16	
(%)	(46.5)		(40.9)		(12.6)	
LATENCY (DAYS)	1.6±.16	≤.01	3.0±.58	≤.01	14.6± 1.6	≤.01
SVAR	23.7%	≤.005	7.7%	≤.005	0	≤.005
C/S	49.2%	NS	32.7%	NS	37.5%	NS
AMNION-ITIS	18.6%	NS	11.5%	<.01	0	<.01
1 MIN APGAR<7	52.5%	≤.005	9.6%	≤.005	0	≤.005
5 MIN APGAR<7	8.5%	≤.005	0	≤.05	0	≤.005

Conclusion: Initial AFI in patients with preterm PROM is predictive of unfavorable pregnancy outcome as defined by shortened latency period, severe variable decelerations during labor, amnionitis, and low 1 and 5 minute Apgar scores. C/S rates were increased in all groups.

313 **DOES "IDIOPATHIC PRETERM LABOR EXIST?"** L. Lettieri, A. Vintzileos, M. Albini[X], M. Martins[X], C. Salafia[X], J. Mead, Univ. of CT Health Center, Farmington, CT.

Williams Obstetrics (18th Edition) states: "In the majority of instances, the precise cause or causes of labor before term are not known." In an effort to elucidate possible causes of preterm labor, we undertook a prospective study of all patients with a singleton pregnancy (23-36 weeks) admitted with preterm labor and intact membranes requiring tocolysis. A comprehensive evaluation plan was instituted including a detailed history and physical examination, targeted ultrasound, amniocentesis for gram stain, culture and glucose, laboratory analysis for infection (CBC, C-reactive protein, urinalysis, cervical, urine cultures) and for antiphospholipid antibody syndrome (ANA, LA, ACA), pathological examination of the placenta, urine toxicology screen and a 12 week postpartum hysterosalpingogram. Thirty consecutive patients who eventually had a preterm birth constitute the focus of this report. The mean gestational age at admission was 29.3 weeks and the mean cervical dilatation was 2.8 cms. The following possible causes of preterm labor were identified: intrauterine infection 14/30 (47%), faulty placentation (abruptio/previa) 12/30 (40%), immunological 10/30 (33%), uterine (uterine anomalies, hydramnios) 6/30 (20%), cervical incompetence 5/30 (17%), maternal (systemic infection, preeclampsia, drug intoxication, etc.) 3/30 (10%), fetal anomalies 2/30 (7%), trauma/surgery 1/30 (3%) and idiopathic 1/30 (3%). Of the 30 patients, 17 (57%) had 2 or more possible causes, 12 (40%) had one cause and only 1 (3%) had no cause identified. As compared to other causes, cervical incompetence and intrauterine infection were associated with a lower mean gestational age at admission and delivery (ANOVA, p<.05). Pregnancy prolongation, gestational age at admission and delivery, birthweight and Apgar scores were not different between patients having one vs. two or more possible causes. We suggest that an exhaustive evaluation can identify possible causes in the overwhelming majority of "idiopathic" preterm labor.

314 **WEST LOS ANGELES PRETERM BIRTH PREVENTION PROJECT (LAPPP): PROGRAM IMPACT.** C.J. Hobel, M.G. Ross, R.L. Bemis[X], J.R. Bragonier[X], M. Bear[X], B. Mori[X], Dept. Ob/Gyn, Cedars-Sinai Med. Ctr. and Harbor-UCLA Med. Ctr., Los Angeles, CA.

The LAPPP is the first prospective randomized controlled trial using a risk scoring system derived from the same population to test education (ED) plus selected interventions. Eight West LA clinics were randomized to form 5 experimental (EXP) and 3 control (CTL) clinics. From Sept. 1983 to Dec. 1988 2084 high-risk patients in the EXP clinics received a program of special ED and frequent visits and each were randomized to a selected intervention (bed rest, psychosocial counseling, oral Provera, a matched placebo and an internal control/special ED alone). There was an 18.8% reduction in preterm births (<37 wks) in the EXP clinics vs. CTL clinics [7.38% vs. 9.09% (p=0.063)]. None of the selected interventions had an effect greater than the internal control/special education alone. However, high-risk patients receiving bed rest had the lowest rates for very low preterm births [<31 wks (p=0.074)]. We believe that the 18.8% reduction in preterm births is due to the overall program effect of special education, frequent visits and the greater attention given patients while applying the selected interventions. Even though the statistical evidence is borderline, the interpretation of these findings should be judged on clinical and biological plausibility and cost effectiveness (see Abstract: Ross, et. al.). Supported by State of California Dept. Health Services, Maternal Child Health Branch.

315 EFFICACY AND SAFETY OF RITODRINE WITH INTRAVENOUS OR INTRAMUSCULAR ADMINISTRATION. Steve N. Caritis, M.D., Karen Leonhard, R.N.[X], Peggy Cotroneo, R.N.[X], Jye Ping Chiao, M.S.[X], University of Pittsburgh, Magee-Womens Hospital, Pittsburgh PA

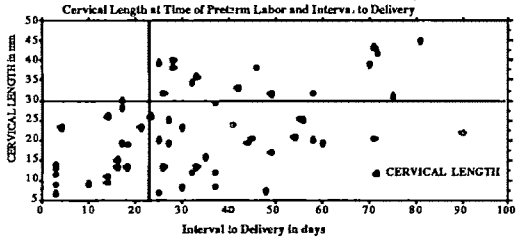
Ritodrine is equally effective in treating preterm labor whether administered intravenously or intramuscularly (Gonik, et al AJOG 159:323, 1988). The two regimens, however, have not been specifically compared in regards to side effects particularly when the dosing regimens are kinetically optimized. We compared these two regimens in 83 pregnant women in preterm labor. The dosing regimens were based on kinetic data in pregnant women. (AJOG 162, p. 429 and 1215, 1990) Significantly, fewer women experienced one or more side effect (SE) with the intramuscular regimen than with the intravenous regimen (p<0.004). Efficacy was comparable in the two groups.

	I.M.	I.V.
SUBJECTS	44	39
With chest pain	2	7
With shortness of breath	5	2
With vomiting	3	6
With heart rate >130 bpm	6	18
With diastolic BP <40mmHg	12	18
With 1 or > of above SEs	18 (41%)	29 (74%)*
With failed therapy	16 (36%)	16 (41%)

I.M. administration of ritodrine offers advantages over I.V.

316 ULTRASOUND ASSESSMENT OF CERVICAL LENGTH (CL) IN PRETERM LABOR (PTL). J Paraskos*, M Waxman*, F Johnson*, J Teteris*, J Iams. The Ohio State University Department of Obstetrics & Gynecology, Columbus, OH

Studies of PTL therapy are complicated by imprecision in diagnosis. We performed transvaginal ultrasonic measurement of CL in 60 women with PTL treated with parenteral tocolysis between 24 - 34 weeks. Measurements were obtained as soon as possible after completion of parenteral medication. Each patient was examined once. Findings were not available for clinical care. Mean gestational age at examination was 31.0 ± 2.6 weeks. Mean CL was 2.2 ± 1.37 mm, range 6.6 - 45.0 mm. CL correlated with interval from admission to delivery(r= 0.48 , p=0.0001,by logistic regression analysis). No patient whose CL was ≥ 3.0 cm had an admission to delivery interval of <21 days. Digital assessment of CL also correlated with interval to delivery (r=0.43, p=0.0006), but was less clinically useful because there was no clinical cutoff to predict low risk of preterm delivery. Ultrasound assessment of CL is useful in PTL to select a group at low risk of preterm birth. Transvaginal scanning may improve the accuracy of diagnosis of PTL.



317 AGGRESSIVE PERINATAL INTERVENTION BASED ON GESTATIONAL AGE DERIVED MORTALITY RATES. L.M.Lopez ,[X] S.B. Effer, M. Whitfield, [X] Division Maternal Fetal Medicine, University of British Columbia, Grace and BCCH Hospitals, Vancouver, Canada.

The aim of the study is to present outcome data, specific to each week of gestation, focusing on weeks 23 to 28, adjusting for potential confounders and with enough power and precision to use in both clinical management, decision making, and counselling patients, appropriately informed. All births between 23 and 28 completed weeks gestation, born in Grace Hospital, Vancouver, during 7-year period from January 1983 to December 1989, were included for analysis. Gestational age errors present in 34.3 % of the patients were corrected by early ultrasound reports available in 88.3% of patients. Neonatal mortality was calculated for each week of gestation (Table I).The effects on neonatal mortality of : fetal sex, use of steroids, premature rupture of membranes (PROM) , and delivery mode in breech presentation were statistically analyzed for each week of gestation. Significant difference was found in : fetal sex at 27 and 28 weeks; PROM at 27 weeks; delivery mode in breech at 25 weeks ; and steroids at 28 weeks. Long term follow up in this population was limited to survivors with birthweight under 800 grams. Major disability decreased in frequency in each week from 23 to 26 weeks ((71.4% to 10.0%) with a dramatic increase at 27 weeks (50%). This , we postulate is due to the fact that 27 weeks survivors who weighted less than 800 grams , have a major degree of IUGR. We conclude that these mortality rates, coupled with known severe degree of disability should deter us from recommending aggressive measures with major risks to mother, with no significant fetal benefit, at gestational ages less than 25 weeks.

Table I - Neonatal Mortality - 1983-1989 Grace Hospital -Vancouver, B.C.

Weeks	total	LVB		SB		NN. SURV.		NN. MORT. RATE
	N	N	%	N	%	N	%	%
23	83	56	67.5	27	32.5	9	16.1	83.9
24	133	108	81.3	25	18.7	44	40.7	59.3
25	184	161	87.5	23	12.5	88	54.6	45.4
26	193	177	91.7	16	8.3	111	62.7	37.3
27	201	188	93.5	13	6.5	143	76.0	24.0
28	231	221	95.7	10	4.3	192	86.9	13.1
Total	1025	911	88.9	114	11.1	587	57.2	42.8

318 **CERVICAL DILATION IS THE BEST PREDICTOR OF RISK FOR PRETERM BIRTH** J. Smeltzer, J. Lewis,[X] P. VanDorsten, D. Cruikshank, Depts. OB/GYN, Medical College of Virginia, Richmond, VA, Washington University, St. Louis MO

Preterm birth is the most important cause of death and disability of the normally-formed infant. Interventions to prevent preterm birth must be able to identify those at highest risk. To evaluate the importance of the factors which can lead to preterm birth, data on social, historical and current pregnancy risk factors were collected from 971 patients with 1067 consecutive pregnancies referred to a special treatment clinic by city public clinics for fixed criteria. Of these, 981 participated at or after 25 weeks, with 63786 days of observation from 25 to 37 weeks. Complete data were available on 973 (99.2%). Cervix exam data were prospectively collected semi-weekly to 34 weeks on outpatients. Intensive treatment was used to 34 weeks. There were preterm labor or ROM in 40.8%, and birth in 30.1%, perinatal death in 10.4 per 1000. The effects of the factors were estimated in a stepwise fashion by a censored regression iterative least squares (ILS) model on cohorts by weeks at exam. Of social, historical and pregnancy scores, only twins had a consistent effect. Cervix dilation at the internal os was a consistent predictor of a shortened pregnancy across gestational ages. Other parts of the exam were much less important, but of expected direction.

Effect of factor on mean interval to delivery

Week, (n)		Dilation	Length	Station[1]	Twins
		days/cm	*days/cm*	*days/score*	*days*
27	(202)	-10.148[c]	5.094[b]	-8.027[b]	-18.218[a]
28	(336)	-5.087[c]	2.283	-0.524	-4.088
29	(313)	-9.164[c]	2.535	-3.570	-20.535[a]
30	(348)	-6.332[c]	2.937[a]	-5.904	-1.658
31	(320)	-7.142[c]	0.103	-2.093	-14.631[b]
32	(355)	-4.623[c]	1.863	-2.404	-5.567
33	(321)	-4.789[c]	1.347	-1.770	-3.147
34	(348)	-4.639[c]	0.552	0.373	-2.683

[1]"High"=0;Minus=1;Engaged=2;Positive=3. p < [a]0.05, [b]0.01, [c]0.001.

319 **EARLY CERVIX DILATION WARNS OF PRETERM LABOR** J. Smeltzer, J. Lewis[x], D. Cruikshank, P. VanDorsten
Depts. OB/GYN, Medical College of Virginia, Richmond, VA, Washington University, St. Louis MO

Effective treatment of preterm labor requires its early recognition. Only when the condition is recognized prior to advanced cervical dilation or rupture of membranes can the labor be effectively arrested. Home uterine monitoring and periodic clinic monitoring are available for this purpose. Their high cost and low specificity make them useful only for high-risk groups. Most preterm births occur in women without prior risk factors. An ideal system would be low cost and identify most of those with truly high risk. Hendricks described an exponential increase in cervix dilation in the weeks prior to labor in term patients. This study addresses the ability of cervix exam to predict new onset preterm labor from 26 to 36 weeks gestation. Cervical exams were recorded semi-weekly in this period from 2070 exams of 842 pregnancies at risk for preterm birth but with no preterm labor in the index pregnancy. Data were analyzed using a regression model for censored data by weekly cohort. Independent variables were social risk score, obstetric history, twins, cervix consistency, station, length, and dilation. Other variables' coefficient estimates were weaker and inconsistent, but cervix dilation had a strong, consistent effect on time to spontaneous labor. Controlled for all other variables, the estimated change in mean time to labor ranged from -5.09 days/cm dilation at 32 weeks gestation to -13.39 days/cm at 27 weeks, with high estimates at early ages (p <.0001). The power of cervix dilation alone to predict preterm labor in this sample was computed by week from exam for exams <31 weeks.

Labor before end of week=>	1	2	3	4
Sensitivity	.603	.524	.457	.419
Specificity	.771	.778	.781	.783
Positive predictive value	.157	.217	.263	.308
Negative predictive value	.965	.933	.893	.858

Cervix dilation predicts most labors within two weeks of exam.

320 **A NEW MODEL FOR PRETERM BIRTH RISK ESTIMATION.**
J. Smeltzer, Dept. OB/GYN, Washington Univ., St. Louis, MO

Clinical studies of preterm birth risk suffer from two problems:

1. Censored observations: patients who withdraw by induction of labor for unrelated reasons or leave the study, because exclusion and inclusion both bias the study results.
2. No statistical model of delivery time to permit both control for the effects of multiple factors and estimation of risk.

The proportional hazards model of Cox (PH) meets the first problem, but can not be used for risk projection. The iterative least squares regression model with censoring (ILS) of Schmee and Hahn estimates risk directly and shows factor effects in days. ILS was tested versus PH in a simulation with three independent factors of prevalences of 50%, 30%, and 10% and effects of -2, -10, and -5 days on a normal random variable with mean = 25 days, S.D. = 7 days, n = 200, over 50 trials. This was repeated with a random censoring of 1% per day. Power of ILS was compared with PH at alpha=.05. Results for percent power and censored mean estimation are:

Effect, prevalence=>		-2 days, 50%	-10 days, 30%	-5 days, 10%
Power	ILS	56%[!]	100%	98%[*]
(No censor)	PH	44%	100%	92%
Power (censored	ILS	60%[!]	100%	98%[!]
1% per day)	PH	36%	100%	86%
ILS Mean ± S.E. d.		-2.132±.153	-10.111±.180	-5.194±.223

[*]p<.05 [!]p<.01, better than PH by exact test

The ILS model is more powerful than PH and not biased under these conditions. ILS assumes a normal survival distribution, and linear effect of the factor. A study population of 1067 pregnancies with risk for preterm birth was tested for these assumptions using interval to delivery by cervical exam of cohorts by weeks gestation at exam. The normal survival model fit the data best of the six tested. Estimates and residuals were normally distributed, homogeneous, and met model assumptions. ILS is ideal for estimating birth risk with censoring during the third trimester, simultaneously controls for multiple risk factors, and yields understandable results.

321 EFFECT OF TIME AND TEMPERATURE ON AMNIOTIC FLUID GLUCOSE CONCENTRATION. W. Meyer[X], D. Gauthier[X], A. Bieniarz, University of Illinois, Chicago, IL.

Amniotic fluid glucose concentration (AFGC) has been used to detect intraamniotic infection. However, immediate analysis of AFGC may not always be practical. The purpose of this study was to determine the effect of time and temperature on AFGC. METHODS. Amniotic fluid was obtained from patients with preterm labor or preterm rupture of membranes who underwent amniocentesis to assess for infection. Twenty samples were kept at 37°C, 13 samples were frozen (-20°C). Baseline and 12 hour AFGC was determined on all samples. AFGC was assessed at 2,4, and 6 hour intervals on unfrozen samples. Aerobic, anaerobic, and mycoplasma cultures were performed on all samples. Paired T-test was used for analysis. RESULTS. In noninfected samples, a decrease of ~7% (1-3 mg/dl) in AFGC was noted over 12 hours at 22°C and 37°C (p=NS). In samples with positive cultures, AFGC decreased ~54% over 2 hours (p=0.016) but then remained stable. AFGC was unchanged from baseline levels in frozen samples regardless of culture results. CONCLUSION. AFGC IN NONINFECTED SAMPLES DID NOT CHANGE OVER 12 HOURS AT ANY OF THE 3 TEMPERATURES TESTED. THIS QUALITY OF AFGC MAY BE HELPFUL IN DIFFERENTIATING NONINFECTED FROM INFECTED SAMPLES.

322 **PREDICTING PRETERM LABOR AND BIRTH**
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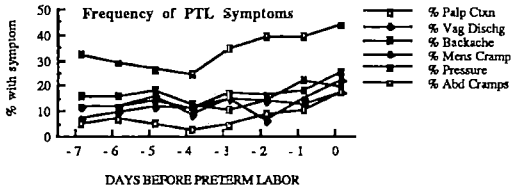
An ongoing prospective study of pregnant women with past preterm deliveries without uterine anomalies or medical problems was initiated in order to develop a possible predictive model for preterm delivery. Creasy scores, cervical cultures, vaginal ultrasounds, weekly uterine activity monitoring, vaginal pH, and cervical Bishop were obtained (20-37 weeks) from 314 weekly observations on 20 patients (8 preterm, 12 term). Preterm patients had 1 ± 1.5 contractions (mean ± standard deviation) initially and 3.3 ± 3.2 prior to delivery (within one week). The initial Bishop score was 2.4 ± 1.8 and 6 ± 3.3 prior to delivery. Full term patients had no contractions initially and 4.5 ± 5.7 prior to delivery. The initial Bishop score was 1 ± .75 and 5.4 ± 2.6 prior to delivery. Differences between cumulative Bishop scores and cumulative contractions in preterm and term groups were statistically significant after 32 weeks gestation. These initial results encourage continuation of the study. Supported by NIH #RR00210-26.

323 PLACENTA PREVIA: DOES PRETERM LABOR CAUSE BLEEDING?
E.F. Magann,[X] C.A. Johnson,[X] K.S. Gookin,[X] W.E. Roberts, R.W. Martin, J.C. Morrison, Dept. Ob/Gyn, Univ. Mississippi Med. Ctr., Jackson, MS

Objective: Determine if an increase in uterine activity (UA) precedes bleeding in patients with placenta previa (PP). Patient Population: This descriptive study involves 22 women > 24 weeks with total PP. Because of other risk factors for preterm birth (PTB) (multifetal gestation, prior PTB due to PTL, etc.), they monitored UA for 2 hours per day and received daily phone contact by perinatal nurses (Tokos Medical Corp., Santa Ana, CA) in addition to regular high-risk care. Monitoring began at 26.3 ± 4.4 weeks' gestation and continued until 37 weeks (or delivery if preterm). Compliance with monitoring requirements, confirmation of PP, and a documented bleeding episode after monitoring began (and before PTL) were inclusion criteria. Main Outcomes Measured: Number of patients with PTL, number of bleeds, early delivery due to bleeding, gestational age at delivery, and UA for the week prior to bleeding. Results: Of the 22 patients, two had documented PTL. All subjects had at least one bleed, 7 women had two bleeds, and 4 subjects had three episodes of bleeding. Only three patients delivered at term while the remainder (86%) delivered preterm. The gestational age at first episode of bleeding was 29.1 ± 3.6 weeks and at delivery was 34.3 ± 3.3 weeks. Nine women (41%) had an increase in UA above baseline the day of or the day preceding the first bleed. The increase in UA was not statistically significant when compared to the 6 days prior to bleeding when all 22 patients were considered. Eleven patients were prescribed prophylactic tocolytic drugs during pregnancy but there was no alteration of UA or in the relationship to bleeding in these subjects. Conclusion: This data suggests that bleeding from PP in some cases may be due to increased UA but may also be associated with thinning of the lower uterine segment and detachment of the anchoring villi without UA.

324 PROSPECTIVE EVALUATION OF SYMPTOMS (Sx) PRECEDING PRETERM LABOR (PTL): J D Iams, M Parker*, FF Johnson*, The Ohio State University Department of Obstetrics & Gynecology, Columbus, OH.

The clinical utility of reported Sx in diagnosing PTL is controversial, based on studies of patient recall. No prospectively collected data about PTL symptomatology has been published. During a trial of an ambulatory contraction monitor, we collected information prospectively about several common Sx of PTL. Data were obtained by daily telephone contact with subjects at risk of preterm birth who had not had PTL in the current pregnancy. Fifty seven women developed PTL. Their answers to a standard battery of yes/no questions for the 7 days preceding their admission for parenteral tocolysis form the basis of this descriptive report. There is a 2 to 3 day period prior to PTL in which several common Sx, (self-detected contractions, vaginal discharge, menstrual cramps and backache), are seen to occur with increased frequency. These Sx are common in normal pregnancy, and are unlikely to be individually predictive of PTL. However, the persistence of these Sx, especially in combination, should prompt an office visit for cervical examination and uterine activity monitoring.



326 **A PROSPECTIVE RANDOMIZED CLINICAL TRIAL COMPARING A NEW ORAL SUSTAINED RELEASE RITODRINE VERSUS CONVENTIONAL RITODRINE TABLETS** Zion Hagay[X], M. Epstein[X], Z. Gotlib[X], C. Goldchmit[X], I. Blickstein[X], A. Mazkel[X], V. Insler[X] and E.A. Reece*, Kaplan Hospital, Rehovet, Israel and *Temple University School of Medicine, Philadelphia, PA

Oral conventional ritodrine (OCR) therapy requires multiple daily doses. A new sustained-release (SR) form of ritodrine was introduced which allows a reduction in the frequency of drug intake. Although, such may improve compliance and efficacy it remains unknown whether this presumed benefit is at the expense of increase cardiovascular side effects. **MATERIALS AND METHODS:** All patients (mean GA: 30.7±3 weeks) admitted to the study had successful IV tocolysis. Patients were randomly assigned to treatment that consisted of either 10 mg of OCR administered every 8 hours (80 mg/day) or one 40 mg SR capsule administered every eight hours (120 mg/day). The first oral treatment course lasted 5 days in each patient and then was switched to a second oral treatment course of the other drug for another 5 days. Every patient underwent non-invasive hemodynamic evaluation on days 4 and 9 of therapy. **RESULTS:** In this ongoing clinical trial, 18 patients were studied. Hemodynamic parameters assessed included: shortening fraction (SF), left ventricle ejection fraction (LV-EF), left atrial diameter (LAD), heart rate (HR) and changes in cardiac axis. Results are presented in Table 1.

Hemodynamic Parameter	During OCR Therapy	During SR Therapy
SF (mm)	0.60 (±0.123)	0.55 (±0.125)
LV-EF (%)	67 (±9.08)	69 (± 5/47)
LAD (mm)	34.4 (±2.5)	31.8 (±2.1)
HR (beats/mm)	108 (±19.2)	104 (±10.2)
Cardiac Axis (degree)	44.0 (±20.7)	46 (±26.0)

* Statistical analysis, t-test for paired samples. **CONCLUSION:** This preliminary results show no significant differences in maternal hemodynamic responses during treatment with the new S.R. form (120 mg/day) as compared to conventional ritodrine therapy.

325 PROLONGATION OF TWIN PREGNANCY WITH MAGNESIUM SULPHATE/SUBCUTANEOUS TERBUTALINE PUMP THERAPY IN THE FACE OF ADVANCED CERVICAL DILATATION AND EFFACEMENT, Robert N. Wolfson, M.D./Ph.D, Sandra K. Winn[X], B.S.N., R.N. Memorial Hospital, Colorado Springs, CO

The literature suggests that tocolytic efficacy is lost when there is advanced cervical dilatation and effacement. Since January, 1989 nine sets of twins have been treated in advanced preterm labor (cervical dilatation ≥ 3 cm. and cervical effacement ≥ 80%) with aggressive acute magnesium sulphate tocolysis followed by subcutaneous terbutaline pump therapy. On admission median cervical dilatation was 3.7 cm. (range 3-5 cm.) Median cervical effacement was 90% (range 80-100%) and median gestational age was 31 weeks (range 27-34 weeks). All patients received 72 hours of magnesium sulphate tocolysis, median maximum magnesium maternal serum level 6.4 mg/dl (range 4.2-9.5 mg/dl) and were transitioned to subcutaneous terbutaline pump therapy, median basal infusion rate 0.106 mg/hr, (range 0.088-0.15 mg/hr). Median duration of pump therapy was 14 days, (range 12-29 days). All patients received betamethasone therapy for enhancement of fetal lung maturity and antibiotic prophylaxis for beta-hemolytic Strep. One patient delivered preterm after discontinuing therapy against medical advice. Of the remaining eight, six (75%) achieved either 37 weeks or mature lung indices on amniocentesis. The two preterm births occurred at 29 and 32 weeks gestation after 12 and 14 days of pump therapy respectively. We conclude from this preliminary experience that twin pregnancies complicated by advanced preterm labor can often be prolonged to fetal lung maturity through the use of subcutaneous terbutaline pump therapy.

327 NIFEDIPINE VS. RITODRINE AS TOCOLYTIC AGENTS. Gustaaf A. Dekker MD PhD*, Karin van Dijk*, and Herman P. van Geijn MD PhD* Dept. of Obstetrics, Free University Hospital, Amsterdam, The Netherlands

The effectiveness of ritodrine in the treatment of premature labour remains controversial. In most patients treated with ritodrine tachyphylaxis develops in 2-4 days probably because of homologous receptor regulation and/or induction of cyclic nucleotide phosphodiesterase activity. Nifedipine is effective as smooth muscle relaxant and at the same time has low toxicity and teratogenicity. In the current retrospective study the results of nifedipine (N = 33) in the treatment of premature labour were compared with a control group treated with ritodrine (N = 36). The 2 groups were comparable with regard to parity, age, and gestational age at initiation of therapy. Nifedipine was found to be considerably more succesful in halting labour than ritodrine. The average (± SD) prolongation of pregnancy was in the nifedipine treated women 36,3 days (SD 42,1) vs. 8.9 days (SD 15,2) in the women treated with ritodrin. In the ritodrine group all patients delivered before 34 weeks' gestation. In contrast in the nifedipine group delivery could be postponed till > 34 weeks' gestational age in 8 patients. Side effects with nifedipine were minimal, patients treated with ritodrine demonstrated well known side effects such as nausea, vomiting, palpitations and malaise. Treatment for a patent ductus arteriosus was needed in 18 neonates in the ritodrine group and in only 6 neonates in the nifedipine group. A prospective study has been initiated to assess the definite clinical value of nifedipine as tocolytic agent.

328 **COMPARING THE EFFICACY OF AGGRESSIVE PRETERM LABOR PREVENTION PROGRAM USE BEFORE AND AFTER THE ONSET OF PRETERM LABOR AMONG HIGH AND LOW-RISK PATIENTS.** A.W. Cohen, I. Forouzan, C. Lindenbaum[X], W. Mulla[X], Dept. Ob/Gyn, University of Pennsylvania Medical Center, Phila., PA

The role of home uterine activity monitoring (HUAM) in the prevention of preterm labor and delivery remains a controversial issue. A retrospective review of 245 patients (divided in 3 groups) was conducted. Initially 156 patients were identified at high-risk for preterm labor (PTL), and were started on a preterm labor prevention program including HUAM. Group A consisted of 46 (29.5%) patients from this high-risk population who developed PTL during the index pregnancy. The remaining 110 (70.5%) patients who did not develop PTL were designated as Group B. Group C consisted of 89 patients who initially were classified as low-risk, but developed PTL, and were enrolled in the "program". The proportion of patients who successfully delivered at >37 weeks of gestation was significantly lower in Group A than Groups B and C (41.3% vs. 70% and 66.3% respectively) (P<0.05). The proportion of patients who delivered at gestational ages ranging 29-34 weeks was significantly higher in Group A than Group B (23.9% vs. 7.3%) (P<0.05). We conclude that despite the application of the "program" including HUAM, a significant proportion of high-risk patients will ultimately develop PTL and eventually deliver at <37 weeks and especially at 29-34 weeks gestation.

329 WEST LOS ANGELES PRETERM BIRTH PREVENTION PROJECT (LAPPP): COST BENEFIT OF HIGH RISK PREGNANCY INTERVENTIONS. M.G. Ross, M. Sandhu[X], R. Bemis[X], S. Nessim[X], J.R. Bragonier[X], B. Mori[X], C.J. Hobel. Dept. of Ob/Gyn, Harbor-UCLA and Cedars Sinai, Los Angeles, CA.

Despite intensive investigation, preterm delivery remains the major cause of neonatal morbidity and mortality. The LAPPP, a randomized, controlled study of preterm birth prevention, achieved an 18.8% reduction in the incidence (9.1%) of preterm, singleton births among high risk patients (31% of patients). To evaluate the cost-benefit of the high risk interventions, maternal and neonatal care data were collected on all preterm deliveries (159) and a random sample of term deliveries (140) from high risk patients in control and experimental clinic sites. Costs were determined for prenatal care, inpatient preterm labor, delivery and postpartum care, and newborn inpatient care. The LAPPP experimental clinics resulted in additional maternal prenatal care ($261) and preterm labor inpatient ($127) costs per high risk patient than the control clinics, although delivery and postpartum costs were similar. Experimental clinic high risk patients had an average cost savings of $1,708 for newborn care (p=0.02) resulting in a net savings of $1,320 per maternal/infant pair. Programs of comprehensive prenatal care and patient education may be highly cost-effective in the prevention of prematurity.

*Supported by State of CA, Dept. Health Services, MCH Branch.

330 PLACENTAL PATHOLOGY AND MATERNAL HEMODYNAMICS IN HYPERTENSIVE PREGNANCIES C Salafia[X], TR Easterling, CA Vogel[X], KC Carlson, DA Brateng. Department Laboratory Medicine, Danbury Hospital, CT, University of Washington, Seattle, WA

Women with high resistance hypertension deliver infants who are significantly more growth retarded than women with high output hypertension. The goal of the present study is to examine the relationship between maternal hemodynamics and placental pathology in hypertensive pregnancies.

Material and Methods: 22 hypertensive and 9 normotensive pregnancies were studied. Hypertension was defined by a sustained dBP >90 mmHg. Hypertension was characterized as high resistance if the total peripheral resistance was ≥ 1150. Cardiac output was measured by Doppler technique. Gross and microscopic villous lesions were determined by blinded observer. The data were analyzed by Fischers exact.

Results:

	Normal	High TPR Htn	Low TPR Htn
Degenerative Knots	1/9 (.001)	11/11	1/11 (.001)
Fibrosis	0/9 (.01)	8/11	3/11 (.01)
Accelerated maturity	0/9 (.05)	4/11	0/11 (.05)

P values represents comparisons with the high resistance group. Placentas from high resistance pregnancies exhibited increased perivillous fibrin, fibrinoid necrosis, X-cell proliferation, and decidua atherosis which did not reach significance with this sample size.

Conclusions: We observed a parallel between placental histopathology and maternal hemodynamics which may provide an anatomical basis for previously observed differences in fetal growth and pregnancy outcome.

331 **INTERLEUKIN 6 LEVELS IN AMNIOTIC FLUID.** **R. Silver,* B. Schwinzer,* J. McGregor, Univ. Colorado Sch. Med, Denver, CO**

Interleukin 6 (IL6) has multifaceted activity in inflammatory and immunological processes. Elevated AF IL6 levels were found in patients with intraamniotic infection (IAI) and preterm labor (PTL) refractory to tocolysis. Because of a possible immune basis for both preeclampsia and intrauterine growth impairment, we measured AF IL6 levels in women with small-for-gestation (SGA) fetuses and preeclampsia, as well as in PTL. Third-trimester AF samples were obtained by amniocentesis, and IL6 levels were determined by an ELISA (AMGEN). Controls were obtained from uncomplicated pregnancies at term. Results:

	N	IL6 Level (pg/ml) mean	Range - pg/ml Standard Error		T-test vs Controls
Controls	**25**	**430**	**160-1352**	**57.2**	**–**
SGA	**8**	**144**	**38-353**	**44.0**	**p = 0.01**
Preeclampsia	**15**	**234**	**73-543**	**38.2**	**p = 0.019**
PTL' < 37	**13**	**625**	**215-1609**	**103**	**p = 0.811**
PTL' > 37	**16**	**299**	**130-515**	**34.5**	**p = 0.098**

'Eventual delivery < or > 37 weeks' gestation. Data were normally distributed. Cases of culture + IAI were excluded. IL6 levels were significantly decreased in patients who delivered SGA fetuses and in those with preeclampsia. Results were not significantly different in patients with PTL. Values in patients with PTL delivering prior to 37 weeks were significantly elevated when compared to patients with PTL responsive to tocolysis (p = 0.0032). Of 11 placentas examined in patients with refractory PTL, 7 (64%) showed histological evidence of IAI. Findings of low levels of AF IL6 in preeclampsia and SGA fetuses may signify an immune alteration which merits further study. We confirm that elevated AF IL6 correlates with PTL and delivery. ELISA determination of AF IL6 may become a clinically useful measurement which should be assessed in future trials.

332 **PREGNANCY-SPECIFIC B1 GLYCOPROTEIN LEVELS IN MATERNAL SERUM AND AMNIOTIC FLUID: PREECLAMPSIA, SMALL-FOR-GESTATION FETUS, AND FETAL DISTRESS.** Silver RM,* Heyborne K, Leslie K, Univ. Colorado Sch. Med., Denver, CO

Pregnancy-specific B1 glycoprotein (SP1), produced by syncitiotrophoblast cells, can be measured in maternal serum (MS) and amniotic fluid (AF). MS SP1 levels have been used clinically to predict spontaneous abortion and small-for-gestational age fetuses (SGA). We assessed MS SP1 levels in 135 patients in the third trimester; 79 had uncomplicated pregnancies and 56 were complicated by preeclampsia, SGA, or fetal distress (FD) in labor. SP1 was also tested in AF samples obtained by genetic amniocentesis from 47 uncomplicated pregnancies and 25 complicated by SGA. SP1 levels were determined by radioimmunoassay (Behringwerke). MS SP1 values for complicated pregnancies were compared with those for normal patients matched for gestational age. Despite trends toward low values in the groups with complications, no statistically significant differences were found. MS SP1 levels less than 80 ug/ml (1 to 1.2 standard deviations below the mean for nl controls) have been used to predict SGA in gestations > 30 weeks. This value ascertained 31% (11/35) of women with preeclampsia, 54% (7/13) with FD, and 37% (3/8) delivering SGA fetuses. AF SP1 levels for SGA perinates were also not significantly different than age-matched normal controls, despite a trend towards low values in the SGA group. An AF SP1 value < 260 ng/ml yielded a sensitivity of 40% (10/25) and a specificity of 89% (42/47) in discriminating SGA fetuses. A discriminatory value < 1000 ng/ml increased sensitivity to 80% (20/25) but decreased specificity to 17% (8/47). These data do not support the use of SP1 values in clinical practice. Conversely, trends toward low values in women with preeclampsia, SGA fetuses, and FD merit further research to elucidate the biological function of this protein.

333 PROSTACYCLIN (PGI2) AND THROMBOXANE (TxA2) PRODUCTION IN FIRST TRIMESTER TROPHOBLASTS: EFFECTS OF ARACHIDONIC ACID (AA) AND ASPIRIN (ASA). E Diss*, A Robinson*, S Gabbe, R O'Shaughnessy, R Reiss, J Moore*, R Fertel*, and D Kniss*, The Ohio State University Hospitals Depts. of OB/GYN and Pharmacology, and Cytogenetics, Children's Hospital, Columbus, OH.

Placental levels of PGI2, a potent vasodilator, and TxA2, a potent vasoconstrictor, are reportedly altered in preeclamptic patients. We developed a model to study PGI2 and TxA2 in first trimester trophoblastic cells. Trophoblastic tissue was obtained via transabdominal CVS from 32 pregnancies at 9-11 weeks gestation. Tissue was first grown in culture for cytogenetic studies for 2-3 weeks. Initially, all cell lines were morphologically consistent with villus core cells. Through altering cell density and passage, the cells became morphologically consistent with cytotrophoblasts. Both cell lines were then exposed to AA and varying concentrations of ASA for 24 hours. RIAs were performed to measure the stable metabolites of PGI2 (6-ketoPGF1a) and TxA2 (thromboxane B2). Core cells and cytotrophoblasts demonstrated statistically significant increases in production of both PGI2 and TxA2 in the presence of AA. The villus core cells produced significantly greater amounts of both PGI2 and TxA2 than the cytotrophoblasts. However, the percent inhibition of prostaglandin production by ASA, was greater in the cytotrophoblasts than the core cells (see table). This model may be a useful tool to study the role of the placenta in preeclampsia.

% REDUCTION IN PROSTAGLANDIN SYNTHESIS

	ASA 1μM	ASA10 μM	ASA 100 μM
CORE 6-KETO	8	17	30*
CORE TxB2	3	7	37
CYTO 6-KETO	37*	47*	74*
CYTO TxB2	18*	45*	64*

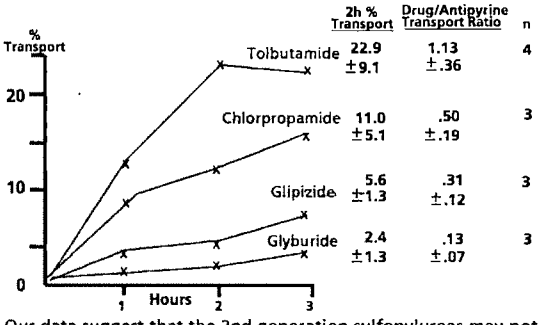
CORE =VILLUS CORE CELL CYTO=CYTOTROPHOBLASTS, *=P<.03

334 **INCREASES IN O_2 TENSION APPEAR TO CAUSE PROSTAGLANDIN-MEDIATED CONTRACTION OF HUMAN PLACENTAL VEINS VIA H_2O_2 GENERATION.** H.A. Omar[X], R. Figueroa, N. Tejani and M.S. Wolin[X], Depts. Physiol. & Ob/Gyn, New York Medical College, Valhalla, NY

Isolated 1-2 mm diameter placental arteries (PA) and veins (PV), obtained from normal-term deliveries, in the presence or absence of endothelium, precontracted with 1-10 nM U46619 to ~2 g of tone, were found to undergo a relaxation of ~250 mg (PA, n=6) and ~400 mg (PV, n=7) when exposed to a Po_2 of 8-10 torr from a Po_2 of 35-40 torr. Reexposure to a Po_2 of 35-40 torr produced a contraction of 466±95 mg in the PV, but not in the PA ($p<0.05$). When exposed to 1-100 μM H_2O_2, the PV produced a contraction of up to 700±190 mg, which was markedly greater ($p<0.05$) than the contraction of 185±59 mg in the PA. Removal of the endothelium did not alter any responses to H_2O_2 or reoxygenation. The contractions to reoxygenation or H_2O_2, but not the relaxation to hypoxia, was eliminated or reversed to a modest relaxation by pretreatment with 10 μM indomethacin, consistent with mediation via the formation of contractile prostaglandins (PG). We hypothesize that reoxygenation may cause a PG-mediated contraction of PV via the generation of H_2O_2 and this mechanism could contribute to vasospasm of the PV.

335 **ORAL HYPOGLYCEMIC AGENTS: PROFOUND VARIATION EXISTS IN THEIR RATE OF HUMAN PLACENTAL TRANSFER.** B. Elliott,[X] S. Schenker,[X] O. Langer, R. Johnson,[X] T. Prihoda,[X] Depts. OB/GYN, Medicine & Pathology. Univ. Texas HSC at San Antonio, Texas.

Our recent finding that the oral hypoglycemic agent glyburide does not significantly cross the human placenta has renewed interest in its use in gestational diabetes. The purpose of this study was to determine whether this unique characteristic is shared by other members of this drug category. The single-cotyledon human placental model was used to compare the maternal to fetal transport of the sulfonylureas. C14-antipyrine was added to these perfusions as a standard reference for simple diffusion. Liquid scintillation spectrometry and high performance liquid chromatography were used to calculate transport from the serial samples obtained during each 3-hour perfusion. The transfer rates below are significantly different (ANOVA, $F<.0003$):



Our data suggest that the 2nd generation sulfonylureas may not reach the fetus in significant levels, and therefore, may be better suited for use in pregnancy than their older counterparts. Further studies to ascertain their clinical utility are required, however.

336 **MATERNAL FLOOR INFARCTION: RELATIONSHIP OF X-CELLS, MAJOR BASIC PROTEIN AND ADVERSE PERINATAL OUTCOME.** **K.K. Vernof,[x] K. Benirschke, G.M. Kephart,[x] T.L. Wasmoen,[x] J.A. Ney and G.J. Gleich,[x] Dept. Ob/Gyn and Immunology, Mayo Clinic, Rochester, MN and Dept. Pathology, UCSD, San Diego, CA.**

To expand our knowledge of pregnancy-associated major basic protein (pMBP) and the X-cell in reproductive physiology, we analyzed pregnancies complicated by maternal floor infarction (MFI). MFI placentas are grossly abnormal showing a striking increase in the number and size of subchorionic cysts and histologically show increased proliferation of X-cells. The pregnancies have poor outcomes with either intrauterine growth retardation or fetal death. Previously, pMBP has been localized to the placental X-cell and identified at elevated levels in serum and amniotic fluid in all normal pregnancies. Here, we present a classic example of MFI along with seven other cases. We analyzed placental tissue, serum, amniotic fluid, and placental cyst fluid. Serum pMBP levels were variably elevated both in normal and MFI pregnancies. Placental tissue from MFI pregnancies had increased numbers of X-cells and fibrinoid material that occupied or surrounded degenerating villi and which stained intensely for pMBP. These results indicate that pMBP, a potent cytotoxin and platelet agonist, is deposited in close proximity to chorionic villi in MFI, and may contribute to the pathophysiology of this disorder.

337 VOLUME REGULATION IN SECOND TRIMESTER CYTOTROPHOBLAST CELLS. Robert S. Egerman,[x] Joan M. Bissonnette,[x] Gail B. Willeke,[x] Dept. Ob/Gyn, Oregon Health Sciences Univ., Portland, OR.

Although ion transport systems have been described in syncytiotrophoblast brush border vesicles, no study as yet addresses cell volume regulation in placental cells. These mechanisms are important in understanding fetal H_2O acquisition in physiologic and in pathologic states (e.g. hyperosmolar ketosis). Cytotrophoblast cells were isolated from 17-19 week human placentas by enzyme digestion and density gradient centrifugation. Video-microscopy captured interval cell images as cells were exposed to various solutions. These recorded images were stored in a MacIntosh computer and later recalled for area measurements using Image 1.22 software. In the continued presence of mannitol (400mM) shrunken cytotrophoblasts increased their area 1.8 to 3.2 fold after an 8-12 minute latency period. When extracellular NaCl was replaced with N-methyl glucamine, the latency period was extended and final volume was depressed. In the presence of the Na^+-H^+ exchanger antagonist, ameloride (100μM), no increase in cell area occurred. Conclusion: 1) Cytotrophoblasts are osmotically active. 2) Na^+ is necessary for regulatory cell volume increase. 3) Na^+-H^+ exchanger is an important system for Na^+ (H_2O) entry into the cell. 4) The latency period suggests that the Na^+-H^+ exchanger must be activated before these cells can regain their volume.

338 **THE EFFECT OF DIFFERENT EXTRACELLULAR MATRIX PROTEINS AND DIFFERENT SERUM-FREE MEDIA ON THE OUTGROWTH OF TROPHOBLAST CELLS ISOLATED FROM HUMAN TERM PLACENTAE**

J. Leyten,* C. Luyten,* R. Pijnenborg* & A. Van Assche

Department of Obstetrics & Gynecology, University of Leuven, Belgium

In order to study the interaction of trophoblast cells with maternal tissue, a monolayer culture system was established. We modified the methods described by Kliman et al (Endocrinology 118,1567,1986) and Loke & Burland (Placenta 9,173,1988). The isolated cells were grown on substrates coated with the following extracellular matrix proteins (ECM-proteins) : Matrigel (Mg.), Collageen IV (Col.), Laminin (Lam.), Fibronectin (Fib.). Serum-free media used : RPMI1640, MEM-D-VAL, Ham's F12, DMEM, K-SFM (GIBCO). Unwanted fibroblast proliferation was evaluated for the different ECM-proteins and media. Immunocytochemical staining of cytokeratin and vimentin was used to differentiate the different cell types in culture. The results are summarized in the table.

Trophoblast outgrowth : + (very low) → ++++ (strong).
Fibroblast proliferation : * (low) → *** (strong).

		ECM - PROTEINS				
		Without	Col.	Mg.	Lam.	Fib.
MEDIA	RPMI1640	+ **	+ *	+ **	+ *	+ *
	MEM-D-VAL	+ *	+ *	+ **	+ *	+ *
	Ham's F12	+ **	+ *	++ ***	++ **	+++ **
	DMEM	++ *	+ *	++ **	++ *	+++ *
	K-SFM	++ *	+ *	+++ *	+++ *	++++ *

Concerning the ECM-proteins, fibronectin, followed by laminin, gives the best result. Matrigel seems to stimulate fibroblast proliferation more than the other media do. For the serum-free media, K-SFM followed by DMEM is more favourable than Ham's F12, MEM-D-VAL and RPMI1640. Ham's F12 seems to stimulate fibroblast proliferation more than the other media do.

339 **SINGLE INTRAUTERINE DEMISE IN TWIN PREGNANCY.** M. Eglowstein and M. D'Alton, Department of OB/GYN, Tufts University School of Medicine, Boston, MA

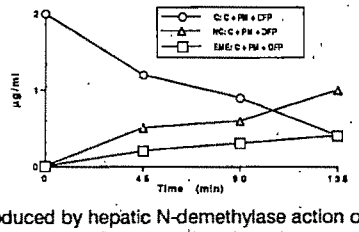
Eighteen twin pregnancies with one intrauterine fetal demise (IUFD) noted after the first trimester were managed using a standard protocol between January 1, 1987 and July 1, 1991, during which time 367 twin deliveries occurred. Of the 17 delivered pregnancies, placentation was determined in 16; 9 were diamniotic/dichorionic (DC) and 7 were diamniotic/monochorionic (MC). Of the DC placentations, 5 cases of IUFD were unexplained, 2 were associated with major fetal anomalies, 1 with nonimmune hydrops and 1 with placental infarct. Of the MC placentations, IUFD in 6 cases was associated with twin-twin transfusion (TTTS); the seventh was associated with preterm rupture of membranes and chorioamnionitis. The interval from diagnosis of IUFD to delivery ranged from 2 days to 11 weeks in DC placentation, and 1 day to 14 weeks in MC placentation. The management protocol consisted of frequent ultrasound for fetal growth, weekly norstress test and biophysical profile after 28 weeks, and weekly coagulation profile. There were no elective preterm deliveries. Incidence of preterm delivery was 82.3% overall (14/17); 7 of 9 DC and all MC gestations delivered preterm. Estimated gestational age at delivery ranged from 28 to 38 weeks in DC gestations and 20 to 36 weeks in MC gestations. No patient required treatment for coagulopathy. 1 case of periventricular encephalomalacia was diagnosed prenatally by ultrasound 24 hours after the death of the co-twin. This seems to indicate that the timing of this neurological insult happened before the death of the co-twin. This report confirms our previous findings of a low risk of clinically significant maternal coagulopathy and a low incidence of periventricular encephalomalacia in co-twins of single IUFD. This series demonstrates a higher incidence of spontaneous preterm delivery in both types of placentation than has been previously reported.

340 **THE DEPENDENCE OF THE PLACENTAL/FETAL RATIO ON FETAL WEIGHT.** R.E. Kurzel, M.D., U.C.L.A./L.A.C. - Olive View Medical Center, Sylmar, CA

Because of the enormous range in placental weights (P-Wt) vs gestation, studies of the placental/fetal weight ratio (P/F) are felt to be more meaningful than P-Wts in predicting the physiologic adequacy of the placenta. Fetal (F-Wt) and P-Wts were obtained from 1633 singleton liveborn pregnancies prepared according to Thomson et al. Mean P-Wt and P/F were determined for 500 gm F-Wt intervals, from 500-6,000 gms, using a fixed F-Wt as the criteria to study the variation in these parameters. P/F's were obtained from the mean F-Wt and P-Wt for each weight interval. RESULTS & CONCLUSIONS: (1) For the entire population, mean F-Wt = 3394 gm, mean P-Wt = 640 gm, and P/F = 0.189 = 1/5.3 (2) Mean P-Wts increase with increasing F-Wts. (3) For a given F-Wt group, the P-Wt follows a Gaussian distribution, with a wide range, ≈ 900 gms. (4) At a given F-Wt, fetal sex does not influence P-Wt. (5) The P/F ratio vs F-Wt shows a decreasing hyperbolic trend, achieving the assymptote (P/F = 0.190) for F-Wts ≥ 2500 gms (term). The leveling off of P/F with F-Wt indicates efficiency achieved at term (minimum in P/F), and supports the concept of Placental reserve.

341 **Metabolism of Cocaine by N-demethylase in Rat Placentae: An Induced Placental Enzyme System.** Bertis B. Little, Ph.D.,+ Daniel A. Roe, B.S.,+ R. William Stettler, M.D.+, Van R. Bohman, M.D.+ Dept. of Ob/Gyn, The Univ. of Texas Southwestern Medical Center, Dallas, Texas.

Previous investigators reported cholinesterase activity (CA) of placenta *in vitro*. Four treatment groups were incubated with cocaine (C) over 4 time periods: placental microsomes (PM) + C, PM + DFP (anticholinesterase) + C, PM + C + butyryl-cholinesterase (BC), and a blank (C only). Gas chromatography was used to quantify C (limit of quantitation-LOQ = 19 ng/ml) and metabolites. BC enhanced C metabolism to ecgonine methyl ester (EME). More than 40% of C was metabolized to NC by rat placenta when DFP suppressed CA (FIG.).



NC is produced by hepatic N-demethylase action on methyl-bearing nitrogen in C, suggesting that placenta as well as liver has this capacity. Hence, this biotransformation of C may be a primary metabolic pathway induced in the cholinesterase deficient placenta. This has clinical implications because NC is 9-fold more active physiologically than C or EME.

342 IS PLACENTAL GROWTH OPTIMAL? LJ Groome, JM Benanti. University of Arkansas for Medical Sciences, Little Rock, Arkansas.

At the present time the relationship between fetal well-being and placental growth is largely unknown, although abnormally small or large placentas are thought to be a marker of fetal compromise. Oxygen (O_2) delivery to the fetus is reduced, because of a reduction in transport area, if the placenta is too small; in addition, the high rate of O_2 consumption by a large placenta can similarly limit fetal O_2 delivery. This relationship between placental size and O_2 exchange raises the question "Is there an optimal placental growth pattern which maximizes fetal oxygenation?" Ordinary non-linear differential equations were derived to describe the axial PO_2 profiles in the maternal (M) and fetal (F) streams for concurrent flow. A Fibonacci search routine was used to assure consistency between umbilical artery and venous PO_2 profiles and fetal O_2 consumption (V_f). Model parameters were well within the range cited for a 3 kg sheep fetus: 1000 ml/min (M) and 500 ml/min (F) blood flow; 18% (M) and 8% (F) flow shunts; 2 ml O_2/min-mmHg placental O_2 diffusivity; data of Edelstone (AJOG, 1985) for V_f; and Hill equations (AJP, 1972) for the oxyhemoglobin binding curves. Based on this mathematical model, we found that O_2 delivery to the sheep fetus is maximized at a placental mass (m_p) of 800 gms. Furthermore, $(\Delta PO_2)UmV/\Delta m_p$ is an order of magnitude greater for placentas weighing <800 mg than for placentas weighing >800 mg (0.04 vs 0.003 mmHg/gm), suggesting that a smaller placenta limits fetal oxygenation to a greater extent. Conclusion: Fetal oxygenation may be an important factor regulating placental growth.

343 THE POST-TERM PLACENTA: SMALL OR NOT? MP Dombrowski, HM Wolfe, AA Saleh, RJ Sokol, Depts of Ob/Gyn and Pediatrics, Wayne State Univ./Hutzel Hosp., Detroit, MI

Placental weight has been directly related to total placentofetal metabolism. Placental growth has been reported to plateau beyond 40 menstrual weeks, with the small, post-term placenta associated with "placental insufficiency". The purpose of this study was to examine placental growth beyond 40 weeks using obstetric estimates of gestational age (GA-OB) based on LMPs, but corrected by fetal ultrasounds and confirmed by Ballard exam, and then compare data based solely on LMP (GA-LMP). Placental weights were obtained from a perinatal database of 33,135 viable, singleton, structurally normal neonates.

	GA-OB		GA-LMP	
weeks	n	weight ± S.D.	n	weight ± S.D.
40	9,648	678 ± 144	4,689	671 ± 146
41	3,538	700 ± 146	3,782	686 ± 153
42	1,353	715 ± 152	2,442	687 ± 154
43	130	781 ± 198	1,287	680 ± 155
44	3	853 ± 103	815	684 ± 150
45	0		578	690 ± 142
46	0		306	691 ± 157
>46	0		356	679 ± 155

Consistent with previous reports based on GA-LMP, there was a plateau beyond 40 weeks; weights at 43 weeks were similar to 40 weeks ($p > .08$). However, when dated by GA-OB, placental weights increased from 40 to 44 weeks ($p < .0001$). The incidence of pregnancies ≥ 42 weeks was significantly less ($p < .0001$) when gestational age was corrected by ultrasound. We conclude 1) typical placental growth continues beyond 40 weeks 2) gestational dating solely by LMP markedly overestimates the true incidence of post-datism and distorts apparent placental growth pattern 3) the concept of placental insufficiency requires re-examination.

Poster Session IV

Friday, February 7, 1992
4:00 p.m.-6:00 p.m.

Grand Salons I-IV

CATEGORIES	POSTER NOS.
Maternal-Fetal Physiology	344-392
Infectious Disease	393-431
OB Anesthesia & Pharmacology	432-435

344 THE EFFECT OF HYPOXIC ACIDEMIA INDUCED BY PLACENTAL EMBOLIZATION OF MYOCARDIAL CONTRACTILITY AND SYSTOLIC TIME INTERVALS IN FETAL SHEEP. R.M. Lewinsky*, R.J. Morrow*, J.W.K. Ritchie. University of Toronto, Toronto, Ontario, Canada.

A study was designed to examine the hypothesis that hypoxic acidemia adversely affects fetal myocardial contractility, thereby causing a prolongation of cardiac systolic time intervals (STI). Fetal sheep (n=7) at 133 days gestation, were studied *in utero* under isoflurane anaesthesia. ECG electrodes were applied to the fetal chest and a 2F catheter-tip pressure transducer (Millar) was introduced through a carotid artery into the left ventricle (LV). Hypoxic acidemia was induced by placental embolization with repeated injections of $5{\cdot}10^5$ 50μM microspheres every 15 minutes via a catheter placed in the fetal abdominal aorta. The LV pre-ejection period (PEP), isovolumetric contraction time (ICT) and ventricular ejection time were continuously measured from the processed ECG and Dopplercardiogram (DCG) obtained with an HP 8040A monitor. Myocardial contractility was assessed by the maximal value of the first time derivative of the ventricular pressure waveform (dP/dt_{max}) corrected for heart rate and preload. A decrease in fetal arterial pH from 7.3 to pH 7.0 was associated with a significant decrease in dP/dt_{max} ($p=0.003$), a prolongation of PEP ($p=0.004$) and ICT ($p=0.002$) as well as with a significant increase in the PEP/VET ratio ($p=0.0002$). We conclude that hypoxic acidemia decreases fetal myocardial contractility, thereby causing a prolongation of the pre-ejection period and the isovolumetric contraction time.

345 THE EFFECT OF CHANGES IN PRELOAD AND AFTERLOAD ON THE CARDIAC SYSTOLIC TIME INTERVALS IN FETAL SHEEP. R.M. Lewinsky*, R.J. Morrow, J.W.K. Ritchie. University of Toronto, Toronto, Ontario, Canada.

Systolic time intervals (STI) are potentially useful for the noninvasive assessment of fetal wellbeing. The effect of changes in cardiac loading on these intervals has not been directly examined in an experimental model. Fetal sheep (n=7) at 133 days gestation, were studied *in utero* under isoflurane anesthesia. ECG electrodes were applied to the fetal chest and a 2F catheter-tip pressure transducer (Millar) was introduced through a carotid artery into the left ventricle (LV). The LV pre-ejection period (PEP), isovulumetric contraction time (ICT) and ventricular ejection time (VET) were continuously measured from the processed ECG and Dopplercardiogram (DCG) obtained with a HP 8040A monitor. Myocardial contractility was assessed by the maximal value of the first time derivative of the ventricular pressure waveform(dP/dt_{max}) corrected for heart rate and preload. Preload was decreased and afterload was increased by brief partial occlusions of the inferior vena cava and the descending aorta respectively, with inflatable balloon catheters in these vessels. Both a decrease in preload and an increase in afterload, caused a significant prolongation of PEP and ICT. ICT is contained within PEP and is the major contributor to its prolongation. VET shortened with both interventions, mostly due to the effect of an increase in heart rate. Myocardial contractility as assessed by dP/dt_{max} did not change during these interventions. This study shows that fetal cardiac STIs are directly affected by changes in preload and afterload, changes which are associated with compression of the umbilical cord.

346 FETAL URINE PRODUCTION IN PREMATURE RUPTURE OF THE MEMBRANES. W. L. Donald, University of Illinois at Chicago, Chicago, IL.

The purpose of this study was to determine if fetal urine production is decreased in premature rupture of membranes (PROM). A 3.5 MHz linear transducer was used to perform serial measures every 5 mins. of the longitudinal (A), transverse (B), and antero posterior (C) diameters of the fetal bladder in 51 patients with PROM between 30 and 34 weeks, 79 patients with intact membranes between 30 and 34 weeks served as controls. Fetal bladder volume was calculated from the formula for an ovoid sphere: $4/3(pi) \cdot [(A/2)\cdot(B/2)\cdot(C/2)]$. Volumes(ml) were plotted against time (mins) and linear regression was used to determine the fetal urinary output (FUO) as m/hr.

RESULTS

	PROM			CONTROLS			P
Gestation	30	32	34	30	32	32	
Mean FUO	10	12	19	16	19	26	0.05
(SD)	(3)	(3)	(6)	(5)	(3)	(6)	
Fill Time	29	34	29	23	27	20	0.05
(SD)	(7)	(6)	(9)	(8)	(7)	(5)	

There was no difference in the maximal bladder volume. CONCLUSION: THERE IS A REDUCTION IN THE FETAL URINARY OUTPUT IN PROM. THE ETIOLOGY REMAINS UNCLEAR.

347 LONGITUDINAL STUDY OF THE AMNIOTIC FLUID INDEX IN POSTDATES PREGNANCY. Ariel D. Marks*, M.S., Michael Y. Divon, M.D. Dept. Ob/Gyn, Albert Einstein College of Medicine, Bronx, New York.

Previous invasive studies of the physiology of amniotic fluid levels in postdates pregnancies used dye dilution techniques and documented a weekly decrease of 28% to 51%. Oligohydramnios was a common finding. Recent sonographic studies of the amniotic fluid index (AFI) in postdates patients indicate a mean weekly decrease of 3% - 12%. The incidence of oligohydramnios in recent studies is unclear. These studies were cross-sectional in design and lacked reliable methods to establish gestational age. Purpose: To prospectively and longitudinally evaluate the change in AFI in postdates. Materials and Methods: Serial AFIs were obtained semi-weekly in 121 well dated (by certain LMP consistent with early sonographic exam) pregnancies > 41 weeks' gestation. Poor fetal testing, oligohydramnios (i.e. AFI≤5.0cm) or a favorable cervix were used as indications for delivery. Results: The AFI increased in 42 patients (35%), did not change in 3 patients (2%) and decreased in 76 patients (63%). The mean AFI at 41 weeks of gestation was 12.4 ± 4.2 cm (±SD). Overall, there was a significant mean weekly decrease in AFI of 25.2% ($p<0.0005$). Oligohydramnios was diagnosed in 13% of these patients. The results of this longitudinal study indicate that in postdates patients:

1. There is a 25.2% weekly decrease in AFI.
2. There is a considerable patient variation in AFI as a function of gestational age.
3. Oligohydramnios is absent in most of these patients with accurate dating.

348 **BETA$_2$-MIMETICS INHIBIT EGF-INDUCED PGE$_2$ SYNTHESIS IN AMNION-DERIVED (WISH) CELLS** *Su, H.-C.[x], *Fertel, RH.[x], *Gabbe, SG., and *Kniss, DA.[x] Departments of *Pharmacology, and *Obstetrics and Gynecology (Division of Maternal-Fetal Medicine), The Ohio State University College of Medicine, Columbus, OH

Studies have shown that prostaglandins (PGs) produced in amnion, especially PGE_2, increase with gestation and with the progress of labor. The processes which regulate the production of these important mediators of labor are not fully understood; however, there is increasing evidence to support the concept that the labor-initiating signals emanate from the fetus. One such signal is epidermal growth factor (EGF), which is derived from the fetal kidney. Therefore, we have investigated the effects of EGF on the regulation of PG production in human amnion-derived WISH cells. Our previous studies have shown that EGF causes a time- and dose-dependent increase in PGE_2 production in human amnion-derived WISH cells. In addition, preexposure of WISH cells to epinephrine inhibits EGF-induced PGE_2 production. Since the β-adrenergic tocolytics used clinically are derivatives of epinephrine, we determined whether terbutaline and ritodrine are also able to inhibit the EGF response. Our results indicate that terbutaline at 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M causes a 16.2%, 27.1%, 31.6%, and 31.4% decrease in EGF-induced PGE_2 production, respectively. Similarly, ritodrine reduced PGE_2 production in response to EGF. The inhibition could be eliminated by a selective β_2-adrenergic receptor antagonist, butoxamine. Since both terbutaline and ritodrine stimulated cAMP accumulation in WISH cells, we conclude that the inhibitory effect of terbutaline and ritodrine on EGF-induced PGE_2 production is mediated via a β_2-adrenoceptor-coupled adenylate cyclase. Our results suggest an alternative mechanism for the tocolytic effects of β_2-mimetic drugs.

349 ENDOTHELIUM-DERIVED NITRIC OXIDE INHIBITION AUGMENTS ANGIOTENSIN II RESPONSIVENESS IN THE PREGNANT RAT HINDLIMB VASCULATURE. R.A. Ahokas,[x] B.M. Sibai, Dept. Ob/Gyn, Univ. Tenn., Memphis, TN

The vascular endothelium modulates constrictor responsiveness by producing locally active vasodilators; e.g., prostacyclin (PGI_2) and endothelium-derived nitric oxide (EDNO). PGI_2 may be responsible for blunting vascular responsiveness to angiotensin II (AII) and norepinephrine (NE) during pregnancy, but the mechanism is unclear. To determine if EDNO plays a role, we measured the concentration-pressor responses to NE (10^{-8} to 10^{-4}M) and AII (10^{-10} to 10^{-6}M) in the hindlimb vasculature of nonpregnant (NP) and term-pregnant (PG) normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats, untreated or infused with N^G-monomethyl-L-arginine (L-NMA, 10^{-4}M) to inhibit EDNO production. Hindlimbs were perfused with Krebs-Ringer (4 ml/min) containing indomethacin (10^{-5}M) to inhibit PGI_2 production. There were no significant differences in baseline perfusion pressure between NP and PG hindlimbs of either strain, and L-NMA had no effect on perfusion pressure. NE induced similar pressor responses in NP and PG hindlimbs of both strains, and L-NMA increased these responses moderately. Pregnancy attenuated vascular AII responses in both WKY and SHR (Table). L-NMA enhanced vascular AII responses of PG, but not NP, hindlimbs of both strains. These results suggest that pregnancy is not associated with generalized vascular refractoriness to all vasoconstrictors and that increased EDNO, not PGI_2, activity is responsible for blunting vascular responsiveness to AII during pregnancy.

Δ Perfusion Pressure (mm Hg) induced by AII (10^{-8}M)

	WKY		SHR	
	NP	PG	NP	PG
Untreated	24.5 ± 2.3	18.7 ± 1.7*	44.8 ± 5.9	30.7 ± 3.2*
L-NMA	25.7 ± 2.3	30.5 ± 5.7†	52.3 ± 6.9	48.8 ± 5.6†

* P<0.05 vs. NP † P<0.05 vs. Untreated

350 **EFFECT OF LOW DOSE ASPIRIN (ASA) DURING PREGNANCY ON DOPPLER WAVEFORM ACROSS THE MATERNAL LEFT VENTRICULAR OUTFLOW TRACT (LVOT) AND AORTA (Ao).** J.C. Veille, R. Hanson, L. Henderson, B. Veille, M. Sivakoff*, Dept. of Ob/Gyn, Bowman Gray School of Medicine, Winston-Salem, NC and Case Western Reserve University, Cleveland, OH*.

Maternal ingestion of low dose ASA (40-80 mg/day) may lead to changes in blood flow across the LVOT and Ao. To test this, 126 pulsed Doppler were done in left lateral decubitus in patients on ASA and in controls. Studies were done at three different gestational groups (GRP): I = 10-20 weeks, II = 21-30 weeks, III = 31-40 weeks. Six cardiac cycles were analyzed and averaged. Results are expressed as X ± SD. ANOVA was done to assess differences.

GRP	$LVOTPFV_N$	$LVOTPFV_{ASA}$	$LVOTCo_N$	$LVOTCo_{ASA}$
I	105.8 ± 22.6*	119.6 ± 24.0*	5.12 ± 1.3	4.89 ± 1.5
II	111.7 ± 18.7	119.1 ± 17.7	5.39 ± 1.2	5.00 ± 2.2
III	112.3 ± 24.1	113.6 ± 20.3	5.44 ± 1.7	5.43 ± 1.7
	*p < 0.05			

GRP	$AoPFV_N$	$AoPFV_{ASA}$	$AoCo_N$	$AoCo_{ASA}$
I	123.4 ± 23	111.9 ± 21	5.53 ± 1.7*	4.37 ± 1.5*
II	129.3 ± 26	130.9 ± 15	5.87 ± 1.8	5.39 ± 2.1
III	129.6 ± 25	123.8 ± 17	5.98 ± 1.9	5.04 ± 1.4
			*p < 0.03	

(Legends: $AoPFV_{N,ASA}$ = Aortic peak flow velocity in normal and ASA GRP [cm/sec]; AoCo = Aortic cardiac output [l/min]; LVOTPV = Left ventricular outflow tract peak flow velocity [cm/sec]). Results: No major differences were observed among the parameters studied in the three GA studied EXCEPT in early. $AoCo_N$ vs. $AoCo_{ASA}$ and $LVOTPFV_N$ vs. $LVOTPFV_{ASA}$ were significantly different. Conclusions: The significant ↑ in LVOTPFV < 20th week in the ASA may reflect an early effect of low dose ASA which is not present as pregnancy advances. The association of ↑ PFV in the LVOT with ↓ AoCo in the ASA group (GRP I) may reflect a smaller LV outflow tract initially in these patients which then normalizes as pregnancy advances. (Supported by NIH Grant HL38296).

351 **EFFECT OF LOW DOSE ASPIRIN (ASA) ON MATERNAL HYPOGASTRIC BLOOD FLOW.** J.C. Veille, R. Hanson, S. Humphrey, M. Sivakoff*, Dept. of Ob/Gyn, Bowman Gray School of Medicine, Winston-Salem, NC and Pediatrics, Rainbow Children's Hosp, Cleveland, OH*.

Maternal systemic effects of low dose oral ASA have not been studied. Patient Population: Twenty six control (normal) patients and seventeen patients taking 80 mg ASA daily from the 12th week on had blood flow of the right hypogastric artery assessed repeatedly during their pregnancy. Patients were on ASA because of previous history of PIH, IUGR, IUFD or positive ANA or anticardiolipins or lupus anticoagulants. Method: The right hypogastric artery was located using a 3 or 5 MZ transducer (UM9 ATL). Pulsed Doppler was placed in the lumen of the artery. Six cycles were analyzed and averaged using a light pen tablet to trace the outer part of the waveform (Digisonics). Analysis was done at four different gestational age Groups (Grp): I-12-18 wks; II-19-26 wks; III-27-34 wks; IV-35-42 wks. Results expressed as X±SEM. An ANOVA with repeated measurements was done to detect significance.

Grp	HRn	HRASA	PFVn	PFVASA	VIn	VIa
I	79±3	77±3	66±3	64±4	142±14	167±22
II	84±2	85±3	61±4	61±5	143±15	194±3
III	88±2	92±4	65±3	68±4	215±22	199±32
IV	78±3	89±3	71±3	67±7	168±26	129±24

(Legends: HRn=heart rates normal (beats per minute); ASA=aspirin; PFVn=peak flow velocity normal (cm/sec); VIn=volume index normal, i.e., volume of blood through the vessel cross-sectional area/min adjusted for body size (ml/min/kg/m^2); VIa=Volume index in ASA group) Results: 1) HRn and HRASA ↑ until the 35th wk; 2) HRn ↓ afterwards. However, in patients taking ASA, HR did not show this ↓; 3) No * in PFVn vs. PFVASA; and 4) No * in vol/unit area was found between the two groups. Conclusions: Low dose ASA has no significant effects on the right hypogastric artery blood flow. (Supported by NIH Grant HL38296).

352 THE PRODUCTION OF INSULIN-LIKE GROWTH FACTORS (IGF) I AND II IN HUMAN PREGNANCY. H. N. Winn, M.D., W.H. Daughaday, M.D.[x](*), B. Travedi, M.S.[x](*). Div. of Maternal-Fetal Medicine, St. Louis U. and Div. of Metabolism (*), Washington U., St. Louis, MO.

While it well-established that the insulin-like growth factors play an important role in postnatal growth, the impact of these substances on fetal growth remains to be determined. In this study, we measured the concentrations of the Pro-IGF-II E 1-21, IGF-II, and IGF-I in the maternal serum (MS), the umbilical cord serum (CS) and the amniotic fluid (AF) during the third trimester of pregnancy. The IGFs and Pro-IGF-II E 1-21 were measured using the radioimmunoassay (RIA) after acid-acetone extraction.

	PRO-IGF-II (Mean ± SD)	IGF-II (Mean ± SD)	IGF-I (Mean ± SD)
MS	120 ± 38	824 ± 550	560 ± 171
CS	232 ± 48	292 ± 46	126 ± 31
AF	933 ± 67	1280 ± 290	78 ± 16

Conclusions: 1) The **AF** concentrations of both **Pro-IGF-II** and **IGF-II** are significantly higher than the paired **CS** concentrations; 2) The concentrations of both **Pro-IGF-II** and **IGF-II** are significantly higher than those of **IGF-I** in both **CS** and **AF**; 3) There is no correlation between maternal levels of **IGFs** and those in the **AF** or **CS**. Speculations: 1) The production of IGFs in the fetal compartments does not appear to depend on maternal IGFs; 2) IGF-II may play a more important role than IGF-I in modulating fetal growth.

353 **ELEVATED LEVELS OF MSAFP & cICAM-1 IN AMNIOTIC FLUID AT 16 WEEKS MAY MARK EARLY INTRAUTERINE INFLAMMATION.** CM Salafia[x], CAVogel[x], JP ezzullo[x], M Lentner[x] E Mainolfi[x], JPBurns[x], EPhillipson[x], RRothlein[x] G Foye, L Silberman[x] DeptLab Med+ Ob/Gyn Danbury Hospital CT, Boehringer-Ingleheim Corp CT, Dept Ob/Gyr Hartford Hospital CT, TRC, Rhode Island Hospital, RI.

Elevated MSAFP concentrations ([eMSAFP]) in mid trimester are associated with increased risk of poor outcome for the structurally normal fetus. We previously dentified an increased incidence of chronic placental inflammation (villitis) in cases of growth retardation associated with midtrimester [eMSAFP]. The hypothesis that chronic intrauterine inflammation is also present in the midtrimester was tested by assaying amniotic fluid (AF) for the circulating form of intercellular adhesion molecule-1 (cICAM-1), a marker of inflammatory responses demonstrated in increased serum levels in the presence of hepatic and cardiac allograft rejection. 40 patients had amniocentes s at 16-17 weeks for maternal age/anxiety with normal [MSAFP]. 20 patients had amniocentesis at 16-17 weeks for evaluation of [eMSAFP] (>2.0 corrected MOM). cICAM-1 assays were performed by ELISA. Non-parametric testing of data gave the following results (mean +/- SE).

AF-[cICAM-1], normal [MSAFP] = 36.8 pg/ml +/-8.0. AF-cICAM-1],[eMSAFP] =132.6 pg/ml +/- 33 ($p<0.001$).

In cases of both normal [MSAFP] and [eMSAFP], [AF-AFP] was not elevated. These data suggest that in cases of midtrimester [eMSAFP] chronic intrauterine inflammation may be present. This could directly cause [eMSAFP] by changing membrane permeability, either in the extraplacental membranes or in villi and would produce [eMSAFP] when [AF-AFP] was normal. Further studies may confirm the utility of AF-[cICAM-1] in the detection of early intrauterine inflammation and potentially for maternal fetal immunopathology.

354 **RELATIONSHIP OF AMNIOTIC FLUID C-PEPTIDE LEVELS TO NEONATAL BODY COMPOSITION**

M.A. Krew, P.M. Catalano, R.J. Kehl, A.Thomas[x], MetroHealth Medical Center, Case Western Reserve University, Cleveland, Ohio.

Amniotic fluid C-peptide levels have been previously shown to correlate with birth weight in diabetic pregnancies. It has been suggested that insulin acts as a fetal growth hormone and that amniotic fluid C-peptide levels correlate with fetal insulin production. The purpose of this study was to examine whether amniotic fluid C-peptide levels have a stronger correlation with neonatal body composition as compared with total birthweight. We hypothesized that fetal insulin production as reflected by amniotic fluid C-peptide would more closely correlate with fetal fat deposition. In order to obtain a sample with a wide range of fetal fat mass 13 women with singleton pregnancies consisting of 9 diabetics (Class A1-2, A2-5, B-1, D-1) and 4 women with normal glucose screening underwent amniocentesis after an overnight fast within one week of delivery. Gestational age range was 37-40 weeks. 3 infants were LGA, 10 AGA, and 1 SGA by normative birthweight data for our institution. C-peptide was measured by RIA. Neonatal body composition was analyzed within 24 hours of delivery with anthropometric measurements. For the total group there was a significant correlation between C-peptide and fat mass (r=.58, p=.038) and % fat mass (r= .59, p=.035) but not with total weight (r=.30) or lean mass (r= .11). The SGA infant (from an A1) had the lowest % fat mass and was clinically growth retarded. Removing it from the analysis strengthened the correlation of C-peptide with fat mass (r= .68, p=.014) and % fat mass (r=.69, p=.014) but not with total weight or lean mass. These data suggest that the level of fetal insulin production affects fetal growth primarily through body fat when adequate substrate is available and that continuation of this study is warranted to determine if this relationship holds for subgroups based on diabetic status and weight for gestational age. Supported by NIH RR-00210 and 22965

355 **CALCIUM METABOLISM IN PREGNANT WOMEN RECEIVING CHRONIC MAGNESIUM THERAPY FOR PRETERM LABOR.**

L. G. Smith, Jr., R. J. Schanler[x], P. Burns[x], USDA/ARS Children's Nutr. Res. Ctr. and Dept. Ob/Gyn, Baylor Coll. Med., Houston, TX.

Because short-term MgS04 affects calcium metabolism in pregnant women , we hypothesized that long-term use of MgS04 would affect mineral homeostasis and bone mineralization adversely. We studied 22 women receiving long-term MgS04 (duration 8 to 66 d, average daily dose 52 ± 10 g/d, mean ± SD) as therapy for preterm labor. A control population (n=31) also requiring a similar degree and duration of strict bedrest for obstetrical indications were enrolled and matched for age (30 ± 3 wk) and delivery (33 ± 4 wk). Weekly serum and urine measurements were obtained for 4 wk. The groups had similar serum concentrations of albumin, osteocalcin, and vitamin D metabolites and urinary excretion of creatinine, phosphorus, and zinc. The following indices differed from the baseline (in the MgS04 group), did not fluctuate during the study interval, and remained significantly different between the two groups:

Average values	MgSO4	Control	p
Serum Mg(mg/dl)	5.2 ± 1.1	1.8 ± 0.2	<0.001
Serum Ca (mg/dl)	7.0 ± 0.5	8.3 ± 0.4	<0.001
Serum PTH (pmol/l)	60 ± 16	48 ± 8	=0.001
Urine Mg (mg/24-h)	245 ± 96	94 ± 67	<0.001
Urine Ca (mg/24-h)	844 ± 313	342 ± 145	<0.001

There were no differences in bone mineral content at delivery. Our data suggest that perturbations in calcium metabolism do not adapt to long-term MgS04 therapy and that large urinary losses of Ca are a concern.

356 **THE EFFECT OF TOCOLYTIC AGENTS (INDOMETHACIN AND TERBUTALINE) ON FETAL BREATHING (FBM) AND BODY MOVEMENTS (FM): A PROSPECTIVE, RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL.** Mordechai Hallak, Kenneth J. Moise, Jr., Noe Lira,[x] Karen Dorman,[x] E. O'Brian Smith,[x] David B. Cotton; Dept. Ob/Gyn; Baylor College of Medicine; Houston, Texas

Since two of the important parameters to evaluate fetal well-being are FBM and FM, an increase in these will influence the assessment of the fetal status and subsequent patient management. **Material and Methods:** Inclusion criteria: Normal, low risk pregnancy, normal level II ultrasound, gestational age 26-32 weeks, and no signs of preterm labor. At the same time of the day, a baseline evaluation of FBM and FM was performed over a period of one hour with continuous videotape recording, using an Aloka 680 (Corometrics, Inc). The patients were then randomized to one of three groups and received a capsule which was previously prepared and coded that contained either terbutaline (5mg), Indomethacin (50mg), or placebo. Three hours later, repeat evaluation of the fetus was performed for another one hour period. Maternal venous blood for glucose level and gases was drawn at the start, middle, and end of each of the evaluation periods. Statistical analysis included ANOVA, ANCOVA, and a multiple comparison procedure (Fisher's LSD). **Results:** Ten patients were enrolled in each group. Baseline demographic data were not statistically different. Pre- and post-treatment glucose, pH, pO_2, pCO_2 were the same. Comparison of the groups with respect to FM showed no statistically significant treatment effect. Indomethacin significantly increased FBM from 20.8 ± 13.1 minutes pre-treatment to 42.2 ± 14.8 minutes post-treatment, while terbutaline significantly increased FBM from 19.8 ± 9.0 minutes to 35.2 ± 12.4 minutes. There was no statistically significant change in the placebo group. **Conclusions:** 1. Indomethacin increases FBM by 103% ($p = 0.003$) and terbutaline increases FBM by 78% ($p = 0.008$) when compared to the pre-treatment value, 2. no significant treatment effect was detected on FM, 3. these findings can affect the way FBM is being used to interpret the fetal status in a patient treated with Indomethacin or terbutaline.

357 **IS FETAL HEART RATE VARIABILITY A PREDICTOR FOR FETAL OUTCOME?** A. Samueloff,[x] M. Berkus, N. Field,[x] L. Ridgway, E. Xenakis,[x] O. Langer. Dept. OB/GYN, Univ TX HSC San Antonio, Texas.

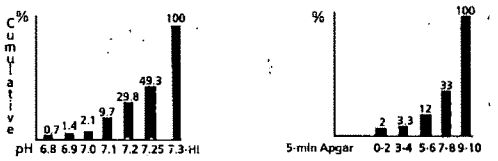
It has become axiomatic that normal variability is an excellent predictor for normal pH and good fetal outcome (FOC). However, paucity of studies evaluated the role of variability as a single predictor for FOC. We, therefore, investigated the hypothesis that normal fetal heart rate (FHR) variability is a single predictor of good FOC. In over 1700 consecutive deliveries, FHR tracings were analyzed. For purpose of analysis, 3 windows of FHR tracings were evaluated: 1) early in labor (30 min); 2) in active phase, 1h prior to complete dilatation (30 min); 3) throughout the entire 2nd stage in segments of 30 min. Variability was calculated by the amplitude from baseline and the number of oscillations in the best 1-min of each 30-min segment. Excluded were twins, fatal malformations and stillbirths. We used variability as the screening criteria (last tracing prior to delivery: normal ≥3) to predict different outcome parameters (with threshold for normality: pH >7.2, Apgar 5 min >7, good outcome - fetus going home after 3-5 days with no NICU admission).

	Sensitivity	Specificity	Positive Pred. Value	Negative Pred. Value
Outcome	9.6%	91.5%	16.5%	91.5%
pH	8.2%	95.4%	25.0%	84.7%
Apgar 5 min	27.3%	95.1%	7.6%	98.9%

Furthermore: 1) infants with low variability had RR of 7.25 for low 5 min Apgar score and 1.85 for pH <7.2; 2) low variability was associated with a 2.1-fold increase for adverse outcome. In contrast, 90% of the adverse outcome was associated with good variability; additionally, 15.2% of the good variability group had a pH <7.2. In conclusion, variability in labor, by itself, cannot serve as a single predictor for fetal outcome.

358 **CAN PH AND APGAR SCORE PREDICT NEONATAL OUTCOME?** A. Samueloff,[x] M. Berkus, N. Field,[x] L. Ridgway, E. Xenakis,[x] O. Langer. Univ. TX HSC at San Antonio, San Antonio, TX.

Although most studies use cord blood gases (CBG) and Apgar score as grouping criteria to evaluate fetal outcome, controversy exists regarding the "cut-off point" for bad outcome. Utilizing immediate neonatal outcome to determine the predictability of Apgars and CBG, values should help resolve the controversy. Therefore, we chose to use a study design that a priori defined 2 immediate outcome groups (good and adverse) and then assessed the characteristics of the corresponding Apgars and CBG. In an on-going study, over 1670 consecutive deliveries were analyzed. Good outcome was defined as newborns going home 2-5 days after delivery with no NICU admission (n=1454). Adverse outcome was defined as newborns admitted to level III NICU with respiratory support or complications (i.e. hypotonia, IVH, neonatal death, sepsis or convulsions [n=144]). Twins, fatal malformations and stillbirths (n=72) were excluded from the study. The cumulative incidence of immediate adverse outcome in relation to 5 min Apgars and arterial cord pH is listed:



In addition: 1) the positive predictive value and sensitivity of pH and 5 min Apgar categories ranged from 0.9%-50% and 11-50%, respectively; 2) in preterm newborns, pH and 5 min Apgars were even less predictive of adverse outcome. Our data show that neither pH nor Apgars can be used as predictors for immediate adverse fetal outcome. In contrast, Apgar score of ≥7 and cord arterial blood pH >7.1 were associated with good outcome in 99.4% and 97.7% of cases, respectively. We conclude that although a good outcome can be characterized by traditional 5-min Apgars and pH "cut-offs," neither Apgars nor pH can be used as predictors for immediate adverse neonatal outcome.

359 SOUND LEVELS IN THE HUMAN UTERUS DS Richards, BS Frentzen,[x] KJ Gerhardt,[x] RM Abrams,[x] ME McCann.[x] University of Florida, Gainesville.

A intrauterine hydrophone was placed transcervically in five laboring women. Sound pressure levels (SPL) were measured in air anterior to the abdomen and compared with intrauterine hydrophone measurements for the following parameters: The pregnant woman speaking, a tape recording of a male voice, and recordings of pure tones ranging from 125 to 10,000 Hz played through loudspeakers. There was a very strong effect of frequency on SPL ($p=0.002$), with enhancement at low frequencies, and attenuation at high frequencies. Intrauterine SPL was very similar to air SPL for the male voice; there was substantial attenuation of the pregnant woman's voice. This study confirms our prior finding in sheep that low frequency sounds readily penetrate into the pregnant uterus.

360 **ARACHIDONIC ACID APPEARS TO CAUSE RELAXATION OF PLACENTAL ARTERIES AND VEINS VIA A CYTOCHROME P-450 METABOLITE.** R. Figueroa, H.A. Omar[X], N. Tejani and M.S. Wolin[X], Depts. Physiol. & Ob/Gyn, New York Medical College, Valhalla, NY

Isolated endothelium-intact (+E) and endothelium-denuded (-E) human placental arteries (PA) and veins (PV) of 1-2 mm diameter obtained from normal term deliveries and precontracted with $PGF_{2\alpha}$ were found to undergo an endothelium-independent relaxation of 40-50% and 60-70%, respectively, when exposed to micromolar concentrations of arachidonic acid (AA). This relaxation (e.g. @ 10 μM AA PA+E = 37±8% (n=7) & PV+E = 61±6% (n=6)), was not altered by pretreatment with the inhibitor of prostaglandin production, 10μM indomethacin (PA+E = 46±9%, PV+E = 59±15%), but was significantly ($p<0.05$) reduced (from PA+E = 49±11% (n=7), PV+E = 65±8% (n=8) @ 10 μM AA) by pretreatment with an inhibitor of cytochrome P450, 30μM SKF525A (PA+E = 26±4% & PV+E = 27±4%), suggestive of AA metabolism via the epoxygenase/monooxygenase pathway. Thus, in HPA and HPV the relaxation to exogenous AA seems to be mediated by metabolites formed via cytochrome P450-linked enzymes. This mechanism could help prevent vasospasm in the placental circulation.

361 **COCAINE METABOLISM DURING PREGNANCY IN MATERNAL, PLACENTAL, AND FETAL COMPARTMENTS: AN *IN VIVO* ANIMAL MODEL.** R. William Stettler, M.D.,[+], Van R. Bohman, M.D.,[+] Donna I. Standard B.S.,[+] K.L. Westfall, M.S.,[+] Bertis B. Little, Ph.D.[+] Dept. of Ob/Gyn, The University of Texas Southwestern Medical Center, Dallas, Texas.

Others have shown rodent fetuses can metabolize cocaine (C) to norcocaine (NC), and human placentae transform C to ecgonine methyl ester (EME). This implies that placentae possess cholinesterase activity (CA), and term rodent (parallel to human second trimester) fetal livers have N-demthylase. EME and NC were not previously quantitated in a single study. It is unknown whether: (1) EME crosses to the fetus, or if (2) the fetus possesses CA. We studied this in rats given C (25 mg/kg) on day 18 of gestation. Four animals were sacrificed at each time period (0, 25, 45, 90, and 135 min. post-IV injection). Maternal and fetal tissues (liver, kidney, brain, heart), placentae, and maternal blood were collected at sacrifice. C, EME and NC levels were analyzed by gas chromatography. C metabolized to NC and EME in the mother, and both were found in placentae. Fetal tissues contained NC and C, but only trace amounts of EME. Fetal liver NC levels increased over time. This implies: (1) fetal liver produces NC and (2) EME and NC only minimally transfer across placenta, perhaps due to high polarity. Thus, C metabolism is different in mother and fetus, likely due to immature fetal enzyme complement. NC in fetus has clinical implications: (1) NC was higher in fetal brain over time, (2) NC is 9-fold more active than C, and (3) fetal brain growth retardation is associated with cocaine abuse.

362 **T CELL DEVELOPMENT IN THE HUMAN FETAL THYMUS: AN IN VITRO MODEL**

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Objective: Current knowledge of T cell development in the fetal thymus has been largely derived from murine studies. Studies of the human fetal thymus has been limited by the difficulty in obtaining specimens. This system was developed to study human fetal thymocyte maturation in vitro. **Methods:** We studied six human fetal thymuses, 18 to 22 weeks gestation. Single cell suspensions were co-cultured with an EBV transformed B cell line as an allogeneic stimulus. Cell surface antigens, CD3 (pan T cell marker), CD4 (helper/inducer), CD8 (supressor/cytotoxic), IL-2R (activation) and T cell receptor (TCR), were analyzed by flow cytometric analysis at days 0, 2, 5, and 7. **Results:** The majority of thymocytes expressed the characteristic double positive (CD4/CD8) precursor T cell form. At this point in development 2.5% of the cells expressed IL-2R and 35% expressed the TCR. After 7 days in vitro the thymocytes lost the immature double positive expression and developed into either CD4+/CD8- or CD4-/CD8+ phenotypically mature T cells. By day 7 expression of IL-2R and TCR was 79% and 90%, respectively. **Conclusion:** Although the cells were not subjected to positive and negative selection which occurs in the thymus before release into the periphery, this in vitro system parallels the in vivo phenotypic maturation processes. This system may prove useful in studying the effects of infectious agents (i.e., HIV) on fetal T cell development.

363 DO PATIENTS WITH A HISTORY OF RECURRENT ABORTION (RA) HAVE SOME UNDERLYING IMMUNOLOGICAL ABNORMALITY? M MacLean[+], R Wilson[+], C Jenkins[+], S H Miller[+], JA Thomson[+], JJ Walker. Departments of Obstetrics and Medicine, Glasgow Royal Infirmary, Scotland, UK.

We have shown that compared to healthy pregnant women, patients with a history of RA have a number of immunological abnormalities. As it is not known whether these changes are triggered by the pregnancy we have investigated 5 patients with a history of RA prior to and following confirmation of their pregnancy. The results show that while there was no significant difference pre and post pregnancy all parameters differed significantly from a group of healthy controls ($n = 25$).

	^{3}H THYMIDINE X 10^3CPM			B CELLS	CYTOTOXICITY
	C	PWM	PHA	CON A	Ig	% 51Cr
PRE PREG	0.3	15.4	27.3	23.2	1373	35.0
POST PREG	0.5	17.0	31.1	24.7	1407	36.8
CONTROL	2.1**	34.5**	52.0**	46.9*	844**	23.2**

** $P < 0.002$ * $P < 0.02$

Mixed lymphocyte reactions carried out between the 5 women and their partners showed activity to be significantly reduced compared to controls (76 + 28 vs 118 + 25% $p<0.03$)), suggesting there are a number of common antigens between the partners. **Conclusions.** These results would suggest that in women with RA there is some underlying immunological abnormality which is present prior to the pregnancy.

364 VENTILATION-PERFUSION MATCHING AND RESPIRATORY ECONOMY DURING MATERNAL EXERCISE. J.M. Pivarnik[X], N.A. Ayres[X], M.B. Mauer[X], B. Kirshon, G.A. Dildy, T. Spillman[X], and D.B. Cotton. Baylor College of Medicine, Houston, TX.

We examined the effects of pregnancy and maternal aerobic fitness on ventilation-perfusion (VA/Q) and physiological dead space-tidal volume (VD/VT) ratios during an acute bout of submaximal exercise. It is possible that these parameters are affected by chronic physical activity if it is continued throughout gestation. Method Seven physically active (PA) and 4 sedentary (SED) women who were tested twice during pregnancy (25 wks, 36 wks) and 12 wks postpartum. Each woman performed cycle exercise for 15 min at a HR of 140 b·min^{-1} (range = 137-142 b·min^{-1}). This insured that relative exercise intensity did not differ between subject groups. Indirect calorimetry was used to measure volumes and fractional concentrations of respiratory gases. End-tidal CO2 (PETCO2) was used as an estimate of alveolar CO2 (PACO2). Cardiac output (Q) was estimated via the indirect Fick method (CO2 rebreathing). Results Exercise metabolic rate was significantly ($P<.001$) greater in the PA (7.2 kcal·min^{-1}) vs SED (4.7 kcal·min^{-1}) subjects, but was not affected by pregnancy status. Alveolar ventilation (VA) and Q responses to exercise were proportionally greater ($P<.001$) in the active subjects which resulted in similar VA/Q in both PA (2.71) and SED (2.63) women. PA subjects had significantly lower minute ventilations per unit of O2 consumed (VE/VO2) compared to SED controls (33.3 vs 39.5). However, VD/VT was less ($P<.01$) in the PA (0.19) than SED (0.24) subjects. Conclusions Pregnancy status did not affect cardiorespiratory parameters as each individual's responses to exercise were similar at both gestational ages and postpartum. These preliminary data indicate that ventilation-perfusion matching during aerobic exercise is not affected by pregnancy status or fitness level. However, physically active gravidas demonstrate more economical ventilation than their sedentary counterparts during cycling performed at similar relative intensities.

365 CAN CIRCULATING CELLULAR FIBRONECTIN PREDICT LABOR ? O. Irion[x], M. Müller Sapin[x], P. Bischof[x], Ph. Extermann[x], F. Béguin. Dept Ob/Gyn, University Hospital, GENEVA, Switzerland

Fibronectin has been isolated from amniotic fluid and placental tissue extracts. Immunohistochemical studies showed its localization to the extracellular matrix of the decidua basalis. Its role as a marker for labor or ruptured membranes has been advocated. Such a marker would be very useful in many clinical situations, especially preterm, and could modify the therapeutic approach. The aim of this study was to evaluate the correlation between cellular fibronectin release into the maternal circulation and imminence of delivery. Methods: 152 patients due to deliver were included in the study from april 19th to may 15th 91. A protocol was completed and 5 ml of native maternal blood was taken. Serum cellular fibronectin was blindly measured (enzyme immunoassay by Adeza Biomedical). Results: Fibronectin was found ≥ 0.1 ug/ml in 32 women (21%), and was absent in the remaining 120. The two groups did not differ with respect to maternal age (29.4 vs 28.5), gravidity (1.7 vs 2.0), parity (0.8 vs 1.0) or gestational age at time of blood sampling (39.7 vs 39.7) or delivery (39.7 vs 39.8). No correlation was found between presence or absence of fibronectin and ruptured membranes (31.3% vs 38.3%), mean temperature (36.5 vs 36.7°C), leucocyte counts (9900 vs 10826/mm3), or presence of uterine contractions (70.4% vs 73.8%). Time measured in hours between blood sampling and rupture of membranes (6.5 vs 2.9), between blood sampling and delivery (10.8 vs 8.2) or between rupture of membranes and delivery (4.4 vs 5.3) was not statistically different for the two groups. Only one patient had preeclampsia (fibronectin < 0.1 ug/ml). Conclusion: we observed serum fibronectin ≥0.1 ug/ml in 21% of our patients. However, this group did not show any difference compared with the other patients. Presence or absence of cellular fibronectin was not correlated with ruptured membranes, uterine contractions, or imminence of delivery. In our hands, this test cannot predict labor and delivery. Further studies may help us to understand the significance of a positive measurement of fibronectin in maternal serum.

366 **MATERNAL SERUM CAFFEINE AND PARAXANTHINE LEVELS AND THIRD TRIMESTER FETAL BIOPHYSICAL ACTIVITY.** **L.Devoe,MD C.Murray RN[x], A.Yossef,MD[x],M.Arnaud,PhD[x], Dept. OBGYN, Med. Coll. Georgia, Augusta, GA; Nestle Research Center, Lausanne, SZ**

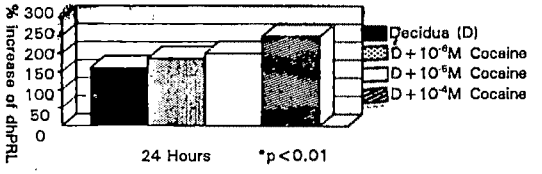
Caffeine and paraxanthine (PX) are found in the blood of most pregnant women and achieve higher concentrations in fetal blood. Their effects on fetal biophysical activity are not well known. We performed 120 2-hour continuous ultrasound observations of fetal heart rate (FHR) and fetal breathing and body movements (FBMs and FMs) in normal 32 - 40 week gestations, standardized for time of day and overnight fasting state. These data were correlated with maternal serum levels of caffeine and PX obtained before and after each study and measured by HPLC.

	Caffeine		PX	
	r	p	r	p
FHR Baseline	-.30	.03	.13	.37
FHR Variation	.12	.38	.002	.99
FBM Incidence	.24	.08	.08	.58
FBM Rate	.47	.004	.25	.07
FM Incidence	.15	.29	.16	.27

Most biophysical variables had weak or insignificant correlations with maternal caffeine or PX levels. The positive correlation of caffeine levels and breath rate agrees with previous neonatal observations. Fetal biophysical testing should not be affected by typical maternal caffeine consumption in normal third trimester pregnancy.

367 THE EFFECT OF COCAINE ON DECIDUAL PROLACTIN SECRETION *IN VITRO*. C.D. Hsu,* H.A. Zacur,* T.R.B. Johnson, Dept. Gyn/Ob, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Cocaine use in pregnancy has been implicated in increasing the risk of premature labor and in decreasing the volume of amniotic fluid. Mechanisms for these activities remain unknown. Prolactin secretion from human decidual tissue has been documented and increased decidual prolactin (dhPRL) secretion associated with lowered amniotic fluid volume and advancement in fetal lung maturation. We have previously reported that the separation of fetal membranes from the decidual exerts a progressive inhibiting influence on prolactin secretion (AFS Abstract P-175, 1991). Consequently, we sought to determine whether cocaine could affect *in vitro* prolactin secretion from decidua. Fresh decidual tissue was obtained at term during elective cesarean section (n=4) and placed in Gey's buffer with varying concentrations (0,10^{-4},10^{-5},10^{-6} M) of cocaine in a 5% CO_2, 95% air incubator at 37°C for 24 hours. At intervals (0,4,24 hrs), a 0.8 ml buffer aliquot was taken and the prolactin concentration determined by enzyme immunoassay. Prolactin concentrations rose after exposure to cocaine at all concentrations. However, only cocaine at the highest concentration (10^{-4} M) after 24 hours produced a prolactin concentration significantly different from the control group ($p<0.01$). This response of decidual prolactin secretion to cocaine may serve as one possible explanation for the diminished amniotic fluid volume and advancement in fetal lung maturation observed in chronic cocaine users.



368 **HYPOTHALAMIC-PITUITARY-ADRENAL AXIS FUNCTION IN THE HUMAN FETUS:** CJ Lockwood, M Alvarez, N Radunovic[x] Mt Sinai School of Med., New York, NY.

In human pregnancy the relationship between the fetal and maternal hypothalamic-pituitary-adrenal (HPA) axes has yet to be established. Therefore, we measured corticotropin-releasing factor (CRF), corticotropin (ACTH) and cortisol (Cs) levels in 104 paired fetal and maternal serum samples obtained at the time of cordocentesis between 18 and 39 weeks gestation. **RESULTS** CRF: Maternal CRF levels [1.54 ng/ml (±1.5)] were significantly higher than levels in either 26 nonpregnant controls [0.17 ng/ml (±0.07); p=0.001] or fetuses [0.34 ng/ml (±0.16) p=0.001]. Maternal but not fetal CRF levels correlated strongly with gestational age (GA) (r=0.73; p=0.001 vs. r=0.304; p=0.9). ACTH: Fetal ACTH increased (r=0.355; p=0.001) while maternal ACTH decreased with GA (r=-0.21; p=0.037). Cs: Both fetal and maternal Cs correlated with GA (r=0.569; p=0.001 and r=0.39; p=0.001). Significant Spearman Rank Correlations between HPA axes hormones in both fetal (F) and maternal (M) serum are presented below (p<0.05):

	CRF-M	ACTH-M	Cs-M	CRF-F	ACTH-F	Cs-F
ACTH-M	NS	--	-0.27	0.3	0.26	-0.24
Cs-M	0.26	-0.27	--	-0.28	NS	0.53
CRF-F	NS	0.30	-0.28	--	NS	NS
ACTH-F	NS	0.26	NS	NS	--	-0.44
Cs-F	0.37	-0.24	0.53	NS	-0.44	--

CONCLUSION: Maternal CRF increased dramatically across gestation, only weakly correlated with maternal and fetal CS and did not correlate with maternal ACTH. Although hormone bioactivity was not measured, these findings are consistent with a placental-derived CRF secretion, independent of feedback inhibition, which "inappropriately" drives maternal ACTH and Cs synthesis. Furthermore a substantial contribution by CRF-stimulated maternal Cs to the circulating fetal Cs pool may be responsible for the strong correlation between fetal and maternal Cs, their correlation with GA and their inverse correlation with declining maternal ACTH levels.

369 **ß-ENDORPHIN CONCENTRATIONS IN FETAL BLOOD DURING THE SECOND HALF OF PREGNANCY.** CJ Lockwood, N Radunovic[x], M Alvarez, RL Berkowitz. Mt. Sinai School of Medicine, New York, NY.

To evaluate changes in circulating ß-endorphin (BEP) concentrations during fetal adaptation to possible intrauterine stress we measured BEP values in paired fetal and maternal blood samples obtained during 81 "uncomplicated" and 18 "complicated" (multiple cord punctures) cordocentesis between 18 and 39 weeks of gestation as well as in 24 term neonatal samples. **RESULTS:** The mean fetal BEP value from the uncomplicated procedure group [90.5 pg/ml (±59.4)] was significantly lower than BEP levels from neonates [228.4 pg/ml (±166.2); p <0.001], and from the complicated procedure group [771.2 pg/ml (±335.9); p< 0.001] but significantly higher than mean maternal values [70.5 pg/ml (±48.8); p< 0.02]. Fetal BEP levels from the uncomplicated but not from the complicated group significantly correlated with maternal values (Spearman rank r=0.47; p< 0.001 vs. r= -0.08; p> 0.5). Fetal BEP levels did not correlate with gestational age. **SUMMARY:** These findings suggest that delivery and fetal adaptation to possible intrauterine stress are associated with significant increases in BEP levels. While a maternal and/or placental contribution to steady state circulating fetal BEP levels can not be excluded, it appears that the fetal pituitary is the primary source of circulating fetal BEP during possible intrauterine stress.

370 **MATERNAL AND FETAL RESPONSES TO ACUTE HEMATOCRIT REDUCTION.** D. Mostello, C. Chalk[x], C. Mack[x], B. Fisher[x], K. Clark[x], Perinatal Research Institute, University of Cincinnati, Cincinnati, Ohio 45267

Anemia was produced by a 2000ml exchange transfusion with plasma/crystalloid in 11 pregnant ewes; 4 underwent exchange transfusion with whole blood. Hematocrit reduction (from 28±2.9 to 17±3.4%) resulted in decreases in arterial O_2 content (O_2C_a)(6.4±0.6 vs 9.8±2.1 ml/dl, anemic vs control mean ± S.D.), uterine O_2 delivery(DO_{2ut})(by -34.8±5.2 vs -17.2±13.5%), mean arterial pressure (MAP)(by -11.3±5.2 vs -2.0±4.7mmHg), uterine vascular resistance (UVR)(by -27.1±7.2 vs -10.1±18.2%), uterine venous O_2C (3.6±0.7 vs 7.0±2.3 ml/dl), fetal p_aO_2(by -11.5±14.5 vs 6.1±10.6%) and fetal O_2C_a (by -23.0±14.6 vs 1.9±21.2%). Maternal heart rate (MHR)(by 15.6±18.8 vs -7.5±12.3bpm), fetal HR (by 12.4±14.6 vs -10.5±14.7bpm) and uterine O_2 extraction (43.1±7.5 vs 29.1±9.2%)increased. By the following day, MAP, UVR and FHR returned to control levels and cardiac output (C O) had increased (by 15.3±15.7 vs -8.8±8.1%). DO_{2ut} remained decreased (by -33.7±11.1 vs -8.4±10.1%). Fetal pH, pCO_2, lactate and hematocrit, maternal pH, pCO_2, pO_2, lactate, and uterine blood flow (UBF) were unchanged. Acute anemia probably results in uterine vasoconstriction as UBF did not increase despite increased C O and decreased viscosity of anemic blood. DO_{2ut} is limited by decreased O_2C and lack of increase in UBF. Despite increased uterine O_2 extraction, the fetus becomes mildly hypoxemic.

371 **EFFECT OF SOUND STIMULATION ON FETAL CEREBRAL METABOLISM AND FETAL OXYGENATION.** C. R. Chao[x], G P. Guy[x], K. E. Jack[x], S. S. Daniel[x], R. I. Stark[x], Dept. of Ob/Gyn, Columbia University, New York, NY

Previous studies have demonstrated that sound stimulation increases glucose metabolism in many regions of the fetal brain. However, the metabolic fate of that glucose has not previously been determined. **Methods:** Near-term fetal sheep were chronically catheterized in brachial arteries and the superior sagittal sinus. Sound stimulation was provided by 1) miniature waterproofed earphones attached to the fetus and 2) an electrolarynx applied to the maternal abdomen. Arterial and venous (sagittal sinus) samples were taken for glucose, oxygen, and lactate concentrations and blood gases prior to and during sound stimulation. All studies took place in the high voltage state. **Results:** Arterial and venous oxygen content and pO_2 were significantly decreased by 6-7% during sound stimulation. The arteriovenous difference for oxygen was unaffected by sound stimulation, whereas that for glucose increased significantly. The glucose:oxygen quotient, an index of the adequacy of oxygen uptake for glucose uptake, increased from 0.88 ± 0.08 to 1.13 ± 0.12 (p<0.01). These findings are consistent with *stimulated* or *aerobic* glycolysis which has been shown to increase brain lactate concentration in other models. No change in lactate arteriovenous difference could be detected, but this may be due to the relative impermeability of the ovine fetal blood-brain barrier to lactate. The metabolic changes were similar during both types of stimuli, but the electrolarynx group alone exhibited a transient increase in arterial blood pressure. Because sound stimulation may adversely affect fetal oxygenation and cerebral metabolism, caution should be exercised in the fetal diagnostic use of sound stimuli. (HD 26600)

372 **PLASMA ATRIAL NATRIURETIC FACTOR AND ARGININE VASOPRESSIN RESPONSES TO INDOMETHACIN IN THE OVINE FETUS.** Martin P.R. Walker MD, Cecilia Y. Cheung PhD[X], Robert A. Brace PhD[X]. Division of Perinatal Medicine, Dept of Reproductive Med., Univ. of California, San Diego, CA.

Prostaglandins have been implicated in the release of atrial natriuretic factor (ANF) and arginine vasopressin (AVP). We hypothesized that indomethacin (ID) would cause a fall in plasma ANF and AVP in the fetus. After a 1 hr control, we gave 0.31 mg/kg of ID i.v. followed by a 0.015 mg/kg/min infusion to 9 near term chronically catheterized ovine fetuses. Hemodynamic data were continuously monitored for 5 hours and plasma ANF and AVP levels determined hourly. During ID infusion, plasma ANF increased from control of 214 ± 58 pg/ml to 701 ± 193 pg/ml at 1 hr then remained at 427 ± 89 pg/ml (ANOVA, $P<0.00001$ after log transformation). Plasma AVP levels rose from 3.1 ± 0.8 pg/ml to 7.4 ± 2.5 pg/ml by 3 hr, to 20.5 ± 11.5 pg/ml at 4 hr, returning to 7.4 ± 2.5 pg/ml at 5 hr (ANOVA, $P=0.0005$). Multivariate analyses revealed that the increases in plasma ANF ($R=0.56$, $P=0.017$) and AVP ($R=0.76$, $P=0.0004$) were associated with changes in fetal arterial pressure but not in blood volume or venous pressure. In summary, our data do not support the hypotheses that ID, at these doses, causes a reduction in plasma ANF or AVP in the ovine fetus. We speculate that 1) The elevation in arterial pressure in response to ID leads to an increase in ANF and 2) The rise in AVP may mediate the increase in arterial pressure. In addition, the observed rise in AVP may explain the clinical observation that indomethacin use in the human fetus leads to oliguria and oligohydramnios.

373 **PREVALENCE OF COCAINE ABUSE IN A TERTIARY CARE CENTER:** M.J. Paidas[X+], M.G. Neerhof, M. Husson[X], R.J. Librizzi. Mount Sinai Medical Center, New York, NY and Pennsylvania Hospital, Philadelphia, PA.

To address the need for routine screening in a urban hospital with 4600 deliveries per year (65% private, 35% service), a cocaine prevalence study was undertaken at Pennsylvania Hospital. The screening was anonymous, and accomplished over 2 months. 441 urine specimens were obtained from pregnant women presenting or being admitted to Labor and Delivery. The urine was tested for benzoylecgonine, a cocaine metabolite, using the enzyme immunoassay, EMIT[R] d.a.u.[TM] Cocaine Metabolite Assay (SYVA Co, Palo Alto, CA)

RESULTS

Population	# patients	(+) Screen	Percentage %
Private	295	2	0.68
Service	132	4	3.03
Unregistered	14	3	21.43
Total	441	9	2.04

Service refers to patients followed in the prenatal clinic at our institution. This group (132) was divided into high risk (23), low risk (103), and teen (6). The number of positive screens were 0,4,0 respectively. Private (295) refers to patients followed by private practitioners (248) and those followed by a maternal fetal medicine practice (47). The number of positive screens in these latter two categories were 2 and 0 respectively.

CONCLUSION The overall prevalence of positive cocaine screening is low at our institution. Routine screening may be beneficial in selected populations including unregistered patients.

374 **THE RELATIONSHIP BETWEEN FETAL PLATELET FUNCTION IN THE THIRD TRIMESTER AND UMBILICAL DOPPLER VELOCIMETRY.** MJ Paidas[X+], MJ Haut[X], A Ludomirsky and RJ Bolognese. Mount Sinai School of Medicine, New York, NY and Pennsylvania Hospital, Philadelphia, PA.

Reduced fetal platelet counts have been associated with abnormal umbilical doppler waveforms. To investigate the relationship between fetal platelet function and umbilical artery doppler velocimetry, we retrospectively compared the results of platelet aggregation, a measurement of platelet function, with the systolic/diastolic (s/d) ratio of the umbilical artery flow velocity waveform. Our previous research in platelet function in the developing fetus suggests that 'normal' responses in platelet aggregation appear in the third trimester. The study population consisted of 10 pregnant women ranging from 28-36 weeks gestational age. Umbilical s/d ratios were obtained in all patients prior to cordocentesis. Platelet aggregation studies using ADP 2×10^{-4}M, were performed turbidometrically using a Sienco Dual Sample Platelet Aggregation Meter (Sienco, Inc., Morrison CO.). 200 microliters of platelet rich plasma were obtained from each fetal blood specimen after centrifugation.

RESULTS:

Gestational age (wk)	S/D	% Aggregation to ADP	Gestational age (wk)	S/D	% Aggregation to ADP
28	2.6	30.5	33	2.9	29.0
29	2.6	5.0	34	1.8	65.0
30	2.7	7.5	34	3.6	42.0
31	3.7	1.5	34	3.2	100
33	2.4	34.5	36	2.3	18

Doppler derived s/d ratio did not correlate with the platelet response to the aggregation agonist ADP 2×10^{-4}M. ($p > 0.05$, $r=0.07$).

CONCLUSION: In this small series, there is an apparent lack of correlation between a measurement of platelet function, namely aggregation response, and umbilical doppler velocimetry. Further studies in platelet activation and methodology are now in progress to verify our initial results of fetal platelet function.

375 SERUM CYTOTOXICITY LEVELS IN PATIENTS WITH SPONTANEOUS AND RECURRENT ABORTION. JJ Walker, M MacLean[+], R Wilson[+], JA Thomson[+], University Departments of Obstetrics and Medicine, Glasgow Royal Infirmary, Scotland, UK.

We have previously shown that there are immunological abnormalities in patients with spontaneous (SA) and recurrent abortion (RA). As the results suggested that these may be triggered by some serum factor we have studied serum cytotoxicity levels in 20 healthy pregnant women, 9 with SA and 20 with RA, by measuring the release of ^{51}Cr from K 562 cells. Serum cytotoxicity levels did not differ significantly between healthy pregnant women and those with SA (25.7± 7.7% VS 23.7±2.9%). Levels were significantly higher in women with RA (33.4±3.2% $P<0.001$) compared to controls and SA. Elevated serum cytotoxicity was also seen in 5 RA prior to and following confirmation of their pregnancy (35±1.9% vs 33.9±3.2%). **Conclusions.** These findings would suggest that the increased cytotoxicity seen in RA is not triggered by the pregnancy. Also it would appear that the mechanism responsible for triggering the immunological changes seen in SA and RA differ.

376 **OBSTETRICAL OUTCOME IN AEROBICALLY TRAINED WOMEN** Stephen R. Carr[1], Marshall W. Carpenter[1], Richard Terry[2X], Ann Lengle[2X], Barbara Haydon[1x]; Brown University/Women and Infants Hospital[1] and Human Performance Laboratory, Miriam Hospital[2]; Providence, RI

Previous studies examining the effect of physical exercise on pregnancy did not randomize or quantitate exertional duration or intensity. The effect of a 6 or 10 week training period during pregnancy on the incidence of PPROM, delivery EGA, bleeding, birthweight, labor length and Apgar scores at 1 and 5 minutes was examined. 38 women were randomized to either a sedentary pregnancy (S) or to either 6 or 10 weeks of training (T)which required 4 weekly sessions of 30 minutes cycle ergometer exercises at 60-70% VO_{2max} beginning at 20-28 weeks gestation. VO_{2max} was determined by an initial incremental exercise test employing a pregnancy-specific formula extrapolating from individual HR/VO_2 data. During exercise training the cycle resistance was increased to maintain exercise heart rate at the initially targeted rate. **RESULTS:**

	C/S	PPROM	Del EGA	Bldng	BW	LoL	A^1	A^5
T	3/16	3/16	39.7±9	0	3458 ±361	12.2 ±11.8	8.1 ±.6	8.9 ±.2
S	2/20	1/20	39.8±7	0	3435 ±427	12.6 ±12.0	7.1 ±1.2	8.5 ±.5
p	.37	.19	.73	-	.86	.93	.07	.40

CONCLUSIONS: Pregnant women participating in a training protocol experienced no difference in obstetrical outcome when compared to a sedentary cohort. Our results confirm the safety of our exercise protocol in evaluating cardiovascular response during pregnancy.

377 **ACTIVITY RESTRICTION TO TREAT HIGH RISK PREGNANCY: A PHYSICIAN SURVEY.** J. A. Maloni[X], A.W. Cohen, I. Forouzan, E. M. Graham[X], Dept. Ob/Gyn, University of Pennsylvania Medical Center, Phila., PA

There are no standard obstetrical protocols for inpatient or outpatient bedrest and reduced physical activity in patients with preterm labor, incompetent cervix, preeclampsia, placenta previa, or preterm rupture of membranes. A survey was conducted to determine patterns of activity restriction/bedrest therapy for these complications of pregnancy at given gestational ages. Questionnaires were sent to 70 Maternal-Fetal Medicine specialists and 200 General Ob/Gyns. They were asked tc select from varying levels of activity restriction treatment that they would prescribe at 20, 24, 28, 32, and 36 weeks gestation. There was no consensus within the obstetrical and Maternal Fetal Medicine community as to when patients should be hospitalized. There was strongest disagreement about the level of activity restriction required for patients with preeclampsia and placenta previa. Maternal-Fetal Medicine specialists tend to hospitalize these patients at an earlier gestational age and are less likely to deliver patients electively at 36 weeks gestation. Only 32% of physicians noted side effects from bedrest and the duration of the side effects ranged from a few days to several weeks. This study concludes that there is no "Standard of Care" across the nation for Maternal-Fetal Medicine specialists or general obstetricians in the treatment of many high-risk pregnancy complications that may require activity restriction.

378 **IN UTERO ETHANOL EXPOSURE INDUCES NURSING DEFICIENCY IN RAT PUPS.** M. Subramanian[x], X. Chen[x], B. Bergeski[x]. Dept. of Ob/Gyn, Wayne State Univ., Detroit, MI.

Prenatal ethanol exposure induces behavioral abnormalities in rats. In the present study, we examined suckling latencies and milk consumption during early (day 6) and mid- (day 10) lactation in prenatally ethanol exposed pups. On day eight of pregnancy, rats were assigned to control (rat chow) or liquid diet groups containing 0%, 17.5% and 35% ethanol-derived calories (EDC). The 0% and 17.5% EDC diets were similar to 35% EDC diet except that maltose-dextrin was substituted isocalorically for ethanol and both groups were pair-fed to the 35% groups. Following delivery, litters were adjusted to eight and transferred to untreated foster dams. On days six and 10 of lactation, pups were removed at 0800h and returned to dams at 1400h. The time taken for the majority of pups to attach to the nipple and start nursing vigorously (suckling latency) and milk consumption were determined. On day six, suckling latency for the 35% group (10.69 ± 1.43 min) was greater ($p < .05$) than control (7.4 ± 0.63) or 17.5% (6.0 ± 0.79) groups. However, on day 10, the suckling latencies among groups were comparable. Milk consumption was lower ($p < .05$) for the 35% group on day six (4.66 ± 0.29, 5.02 ± 0.52 and 3.46 ± 0.40 gm) and on day 10 (7.97 ± .42, 7.17 ± 0.77 and 5.14 ± 0.63 g for control, 17.5% and 35% respectively). Pups exposed to 35% EDC weighed less up to weaning. These results illustrate the continued nursing difficulties offspring experience following prenatal alcohol exposure. (Supported by NIAAA AA07670).

379 THE MITOGENIC ACTIVITY OF SERUM AND LYMPHOCYTES FROM PATIENTS WITH RECURRENT ABORTION (RA). R Wilson±, M MacLean+, JA Thomson+, JJ Walker University Departments of Medicine and Obstetrics, Glasgow Royal Infirmary, Scotland, UK

We have shown that peripheral blood lymphocytes (PBL) from women with a history of RA have an impaired response to mitogenic stimulation. The aim of this study was to determine the mechanism responsible. Serum and PBL were obtained from healthy pregnant women and from RA. The PBL were made to a standard concentration in RPMI + serum from both patient groups. The PBL were then incubated + mitogens. The activity was determined by ^3H thymidine incorporation. The results (Table) showed significantly greater incorporation when PBL from healthy pregnant women were incubated with their own serum. The response was significantly reduced when PBL from RA were mixed with RA serum.While PBL from the control mixed with RA serum or vice versa gave a reduced response this was not always significant.These results would suggest that PBL from RA are unable to respond maximally to mitogenic stimulation and that this is due, at least in part, to some serum factor.

^3H THYMIDINE (CPM X 103)

	C	PWM	PHA	CON A
N PBL + N SERUM	1.8	208	289	206
N PBL + RA SERUM	2.1	110**	242	183
RA PBL + N SERUM	1.8	152*	259	211
RA PBL + RA SERUM	2.3	141*	158**	140
	P <0.001	P < 0.06		

Results are the mean of 5 experiments

380 FETAL HEMOLYTIC ANEMIA AND FETAL GROWTH.
AR Gregg+ and CP Weiner. Dept OB/GYN, U. of IA, Iowa City, IA.

Products of hemolyzed blood inhibit the action of insulin in vitro (Steinke *Blood 30:359-63; 1967*) and hyperinsulinemia has been observed in fetuses with hemolytic anemia (Brown *Am J Obstet Gynecol 131:682-86; 1978*). It was recently reported without explanation in a small number of alloimmunized pregnancies that the normal maternal-fetal glucose gradient was absent (Nicolini *Am J Obstet Gynecol 161:924-27; 1989*). We hypothesized that hemolytic fetal anemia as defined by gestational age dependent norms (Weiner *Am J Obstet Gynecol 165:546-53; 1991*) would be associated with intrauterine growth retardation (IUGR). 22 fetuses with hemolytic anemia who received 0 (15/22) or 1 (7/22) transfusion after 33 wks gestation were identified from our database. Fetal intravascular transfusion was performed when the hematocrit fell below 30%. Serial sonographic measurements were available for 15 pregnancies. Scattergrams of the data points for estimated fetal weight percentile (EFW), fetal abdominal circumference percentile (AC), and birthweight percentile (BW) were constructed. In all cases, the initial AC was < the 50th percentile ($p<0.001$ from expected distribution) at the time of the first sonogram and diagnostic cordocentesis (26.9wks±5wks). In 12/15 (80%), the AC was ≤ 50th percentile ($p=0.05$ from expected) at the last scan. EFW was evenly distributed at both the initial scan and the last scan. BWs were available from 11 neonates; they were evenly distributed. The fetal AC strongly reflects fetal liver size. The low ACs are consistent with inactivation of insulin by products of hemolysis and the reported absent maternal-fetal glucose gradient. Rather than a loss of glycogen from the liver, these findings more likely represent failure of the fetal liver to perform glycogenesis normally in the presence of hemolysis. CONCLUSIONS: ***The growth limiting effects of fetal hemolytic anemia appear confined to the liver, a reticuloendothelial organ of the fetus. Hemolytic fetal anemia is not a risk factor for IUGR when defined by BW.***

381 THE NONVERTEX SECOND BORN TWIN: INFLUENCE OF PRESENTATION AND MODE OF DELIVERY ON APGAR SCORES AND UMBILICAL BLOOD GAS DATA. SA Ordorica*, IA Hoskins, J Blackstone*, F Inamoratti*, BK Young. NYU School of Medicine, Division of Maternal-Fetal Medicine. New York, NY 10016.

The optimal mode of delivery for the nonvertex second born twin remains controversial. Several studies have demonstrated increased perinatal compromise for the vaginally delivered nonvertex second twin, while other investigators have not shown such an association. A prospective study was therefore undertaken which examined 166 sets of twins, 69 of which included a nonvertex second born. Using umbilical blood gas data and Apgar scores as indices of neonatal condition, oxygenation and acid-base status, vaginal and cesarean delivered nonvertex second born twins were compared by means of the student t-test. No significant differences in either Apgar scores or in umbilical blood gas parameters related to the mode of delivery were noted. A similar analysis of nonvertex and vertex second born twins delivered vaginally also showed no significant differences in these parameters due to the type of presentation. In conclusion, increased perinatal compromise for the vaginally delivered nonvertex second born twin due to mode of delivery is not reflected in either Apgar scores or in umbilical blood gas parameters. Also, there is no significant difference in these parameters related to presentation in vaginally delivered second born twins.

382 AMNIOTIC FLUID ERYTHROPOIETIN IN THE SMALL FOR GESTATIONAL AGE FETUS. T.C.C. Peng, S.McCoy[X] DEPT OB/GYN MEDICAL COLLEGE VIRGINIA/VCU, RICHMOND, VA.

Differentiating the growth retarded fetus (IUGR), at increased risk of adverse perinatal events, from one that is constitutional small for gestational age (SGA) is difficult by ultrasound but a common problem when a SGA fetus is detected by ultrasound biometry. Percutaneous umbilical cord blood studies demonstrate a reduction of pO2 in SGA vs appropriate weight for gestational age (AGA) fetuses. Reduction in pO2 has been shown to elicit fetal specific increased erythropoietin (EPO) production measureable in fetal blood and amniotic fluid (AF). Therefore EPO may be useful in discriminating the SGA fetus that is hypoxic and probably IUGR. This preliminary study investigated the efficiency of AF EPO in discriminating AGA from SGA fetuses, using a commercial RIA kit (EPO-TRAK by Incstar). This kit has a sensitivity of 4.4 mu/ml. AF was obtained in 20 pregnancies prior to labor as labor can increase EPO levels. AF EPO was measured in 4 discrete groups, term AGA and SGA and preterm AGA and SGA. In term AGA (5) fetuses the AF EPO was 4.26±4.4 mu/ml vs 40.5±72.6 mu/ml in term SGA (4); and 8.5±4.3 mu/ml in preterm AGA (4) vs 62.3±123 mu/ml in preterm SGA (7) fetuses. The mean AF EPO level was 8-10 fold higher in SGA then AGA fetuses. However, the sample sizes were small and differences between the groups were not statistically significant. In summary, EPO may be useful as a prenatal test to discriminate AGA vs SGA and IUGR vs SGA but larger sample sizes are needed.

383 **AMNIOTIC FLUID PLATELET FACTOR 4 AND BETA-THROMBOGLOBULIN DO NOT PREDICT LUNG MATURITY.**
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Platelet activating factor (PAF) has been identified in human embryos and fetuses. Possible sources are fetal lungs, kidneys and amniotic membranes. Cultured human fetal lung tissue showed time-dependent increase in PAF associated with accelerated glycogenolysis which may supply energy, glycerol and acetyl Co-A needed for surfactant synthesis (Hoffman et al, 1986). Platelet factor 4 (PF4) and beta-thromboglobulin (BTG) are unique markers of irreversible platelet activation and cleared by endothelium and kidneys respectively. If *in vivo* fetal PAF production increases with gestational age, fetal platelets will become more activated as term approaches and levels of fetal platelet activation products should also increase and correlate with surfactant production. To study this potential relationship, we measured PF4 and BTG by ELISA in 78 genetic amnios and 35 pulmonary maturity amnios. Results were analyzed by Mann-Whitney U test.

	Genetic Amnio	Maturity Amnio	p
PF4 (IU/ml)	1.30±2.34	0.24±0.36	<0.001
BTG (IU/ml)	18.2±13.8	8.55±14.12	<0.001

Using multiple regression PF4 and BTG did not predict PG%. We conclude: 1) the 3rd trimester drop in PF4 and BTG may be due to increased fetal endothelial and kidney maturity, diminished fetal platelet responsiveness to PAF, decreased transudation in fetal skin or other factors regulating platelet function. 2) This pattern, opposite to maternal increase PF4 and BTG with gestational age, support fetal origin, and 3) amniotic fluid PF4 and BTG do not predict lung maturity.

384 NORMALISATION OF GLUCOSE TOLERANCE IN ADULT OFFSPRING OF DIABETIC PREGNANT RATS BY ISLET TRANSPLANTATION IN THE MOTHER.

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Our previous work clearly shows that streptozotocin-induced diabetes in the pregnant rat has long-term consequences for the offspring. This effect is not of genetic origin, but is due to metabolic adaptations induced in the fetus by the diabetic intra-uterine milieu.

In the adult offspring of mildly diabetic mothers the insulin-secreting B-cells are affected ; in the adult offspring of severely diabetic mothers both insulin secretion and peripheral insulin-resistance are involved.

A definite conclusion can be made by normalizing the diabetic state during pregnancy using islet transplantation. Islets were obtained from normal neonatal Wistars after pancreatic digestion with collagenase. Severely diabetic rats received an injection of 2000 islets into the portal vein at day 15 of gestation, sham-transplanted diabetic animals received the solution medium only.

Glycemia was normalized in the transplanted mothers from the day after transplantation and throughout further gestation and lactation.

At weaning (age 20 days) the weight of the pups from transplanted mothers was normal while it was seriously decreased in the sham group (32 ± 2 versus 18 g. ± 1.5), as it was in the previous untreated severely diabetic group.

In the adult offspring of transplanted mothers, insulin and glucose levels during glucose infusion were normal, however plasma insulin levels were increased in the offspring of sham-transplanted mothers (87 ± 14 uU/ml versus 187 ± 21 uU/ml) after a 3 hour glucose infusion. The values of the sham treated group were identical as previously shown in the offspring of untreated diabetic mothers.

385 PLASMA PROTEINS AND NUTRITIONAL STATUS IN PREGNANCY

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Albumin (AL), prealbumin (PA), and retinol binding protein (RBP) are used as long, mecium and short-term markers for protein nutriture. Their serum half lives in the non-pregnant state are 18, 2, and 0.5 days, respectively. We measured the proteins at 18 and 30 weeks gestation and correlated the levels with birthweight and fetal growth retardation (FGR). We also assessed these protein levels in relation to maternal age, race, infant sex and various measures of nutrition status such as hematocrit, height, pre-pregnancy weight, body mass index (BMI), lean body mass, and weight gain. Serum samples were obtained from 289 indigent multiparous women, 29% of whom gave birth to a newborn with FGR. None of the protein levels correlated with FGR. AL levels correlated inversely with birthweight at 18 weeks gestation ($p=0.05$), but not at 30 weeks and neither of the other protein levels correlated with birthweight. At 18 and 30 weeks, there was an inverse association between AL and both pre-pregnancy weight and BMI ($p<.01$), but not with maternal weight gain during pregnancy. AL levels were not related to height or lean body mass. AL levels correlated positively with PA ($p<.0001$) and RBP ($p=.001$) at both 18 and 30 weeks. Hematocrit, infant sex, age, and race did not correlate with serum AL. PA, a protein which migrates during electrophoresis in front of AL but is otherwise not related, also correlated ($p<.01$) with pre-pregnancy weight and BMI at 18 weeks but not with any other factor. PA and RBP levels were highly correlated ($p<.0001$) since they are secreted in association with one another from the liver. RBP and AL levels decreased from 18 to 30 weeks, but for each protein, there was a positive correlation between the 18 and 30 week values ($p<.001$). RBP did not correlate with any of the factors studied except maternal weight gain ($p<.03$). During pregnancy, plasma protein concentrations are regulated by complex and incompletely understood factors. Extrapolating assumptions of nutritional status based upon normal nonpregnant levels of these proteins may not be valid for predicting nutritional status in pregnancy.

386 DOES THE PRESENCE OF TRACE PHOSPHATIDYL GLYCEROL (PG) INDICATE NEONATAL LUNG MATURITY?

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The presence of trace PG obtained on amniocentesis is usually an indication for further testing (Lecithin/Sphingomyelin-L/S). We questioned whether trace (tr) PG in any specific patient indicates lung maturity. 58 nondiabetic patients who underwent amniocentesis from August 1989 to March 1991, delivered within 72 hours of the test, and had L/S analysis were included. Lung maturity was examined by clinical diagnosis and by L/S >/= 2. All patients were < 36 weeks. 8 infants (14%) developed respiratory distress, 5 developed transient tachypnea (overall incidence 24%). 3/11 with L/S >/=2 required oxygen >6 hrs in NBICU (33,33,35 wks). The presence of tr PG predicted mature L/S in 76% (19/25) of patients over 34 wks, but only 52% (17/33) of patients under 34 wks. Conclusion: Tr PG is not a good predictor of pulmonary maturity regardless of gestational age.

387 THE EFFECT OF MATERNAL INTRAVENOUS GLUCOSE ADMINISTRATION ON FETAL ACTIVITY.

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Fetal hyperglycemia has been shown to cause marked stimulation of the fetal metabolic state, resulting in increased oxygen consumption. Bocking, et.al.demonstrated increased fetal breathing movements but no change in gross body movements after intravenous glucose injection (AJOG 1982; 142: 606-611). Others have suggested that increased fetal activity may play a role in this increased oxidative metabolism. The following study was designed to prospectively evaluate fetal activity during maternal intravenous glucose tolerance testing (IVGTT). Fourteen women, 30.7±3.0 weeks gestation, were evaluated continuously for fetal activity with a doppler fetal activity monitor (Toitu Model MT-320).Baseline monitoring began 10 minutes before a fasting blood sugar was obtained. A 25 g load of 50% dextrose was administered and maternal plasma glucose levels were drawn at 15, 30, 45 and 60 minutes via an in-dwelling venous catheter. Six control patients, 29.8±4.8 weeks gestation, were continuously monitored and corresponding plasma glucose levels drawn. However, controls did not receive intravenous dextrose. The plasma glucose levels remained stable in the control group and the corresponding fetal activity was random. Fetal activity in the IVGTT group increased over time and was best characterized by a polynomial regression curve ($y= 16.1 + 0.6X - .004X^2$; $p = .0001$). The increase in fetal activity in the IVGTT group corresponded to the initial rise in maternal plasma glucose levels. Glucose is the principle source of energy for the fetus and crosses the placenta rapidly via facilitated diffusion. Fetal glucose levels correlate well with maternal plasma glucose (100 mg/dl maternal concentration = 80 mg/dl fetal concentration). This study confirms that increased fetal activity is a likely consequence of hyperglycemia, suggesting an association between fetal metabolic state and activity.

388 FETAL BEHAVIORAL STATE AND SWALLOWING RESPONSES TO ORAL WATER. A. Dodd[X], C. Agnew, Y. Fujino, M.G. Ervin[X], M.G. Ross, Dept. of Ob/Gyn, Harbor-UCLA Med. Ctr., Torrance, CA.

Fetal swallowing is a major route of amniotic fluid resorption. Although absent swallowing may result in excess amniotic fluid, previous studies have suggested an increased rate of fluid exchange and fetal swallowing in polyhydramnios. To determine if fetal swallowing is influenced by increased accessibility of fluid, seven ovine fetuses (127±2 d) were chronically instrumented with vascular catheters, fetal electrocortical (ECoG) and esophageal electromyogram electrodes, an esophageal flow probe and a sublingual catheter. Following a 2 h control period, distilled water (22°C) was infused sublingually at 10 and 20 ml/kg/hr for 2 hours each. In response to the sublingual water infusions, fetal plasma NA (142±0.5 to 140.8±0.8 mEq/l) and osmolality (302±2 to 298±2 mOsm) decreased significantly while fetal low voltage ECoG (42±3 to 56±5%) increased. Fetal swallowing rate (47±9 swallows/hr), esophageal flow (19.2±4.2 ml/hr), arterial blood gases, blood pressure and heart rate did not change. These results suggest (1) fetal plasma may be affected by the composition of swallowed fluid and (2) exposure of the fetus to altered amniotic fluid composition and temperature may influence fetal behavioral state, though not swallowing activity.

389 NONINVASIVE CARDIAC OUTPUT IN NORMAL AND HYPERTENSIVE PREGNANCY. GJ Gilson, JF Smith, LB Curet, LA Izquierdo, MS Chatterjee, GO Del Valle[x], GM Joffe[x]. University of New Mexico Hospital, Albuquerque, New Mexico

The objective of the current study was to confirm or refute the hypothesis that the pathophysiology of preeclampsia (PIH) is abnormally high cardiac output (CO). Methodology entailed study of 16 women diagnosed as pure PIH and 22 women with normal pregnancy, all in the third trimester. Hemodynamic data were obtained by pulsed doppler and 2-D echocardiography. All were studied in left lateral decubitus, and hypertensive patients were studied prior to any therapeutic interventions.

Results: (mean+SE)	n	HR (bpm)	SV (ml/min)	CO (L/min)	MAP (mmHg)	TPR* (dynes/sec/cm-5)
Control	22	92±3	113±8	10.5±7	69±2	583±41
PIH	16					
PO	9	84±3	120±11	10.0±.8	103±3	883±85
P1+	7	79±7	121±9	8.9±.3	107±4	966±5
p		.019	NS	NS	.0001	.001

*Heart Rate (HR), Stroke Volume (SV), Mean Arterial Pressure (MAP), Total Peripheral Resistance (TPR), Nullipara (PO), Multipara (P1+)

Conclusions: Patients with normal pregnancy outcome and those with PIH both had elevated CO, which however were not significantly different. MAP was significantly elevated in PIH on the basis of elevated TPR. While this data is consistent with the classic theory of the pathophysiology of PIH, these patients were studied when they already had clinical manifestations of the disease and could have already crossed over into a state of elevated TPR.

390 EFFECT OF MODE OF DELIVERY ON LYMPHOCYTE SUBSETS IN FULL TERM NEONATES. R.Samelson, D.Larkey[X], K.S.Amankwah, P.McConnachie[X], Southern Ill School of Med., Springfield, Il.

With the aid of monoclonal antibodies, immunofluorescence, and flow cytometry, cord blood lymphocyte populations were studied in neonates, 6 delivered vaginally (V) and 6 by cesarean section without labor (C/S). These lymphocyte subsets, or phenotype frequencies (PF), were compared to normal adult values (A) and to each other (2 tail student t test, p less than 0.05). A statistically significant difference did occur in the PF of helper T cells (C) (CD4), more mature B C (CD21), Natural killer C (CD16), and Killer C (CD56). Total T C (CD2) were decreased in V when compared to C/S. The PF of common thymocyte (CD1) co-expressed with mature T C (CD2), suppressor cytotoxic C (CD8), B cells (Dr), early intermediate B C (CD19), and activated T C (CD3/Dr) were the same in all neonates regardless of mode of delivery and were no different from the PF in A. Thus a profile of elevated T and helper T C and depressed Natural killer C is characteristic of C/S; a profile of depressed T C and helper T C and elevated Natural killer C is characteristic of V. Depressed intermediate B C is common to all neonates compared to adult normal values.

391 THE FETUS DURING MATERNAL FEVER: BIOPHYSICAL EFFECTS ASSOCIATED WITH VIRAL SYNDROMES DIFFER FROM THOSE ASSOCIATED WITH PYLEONEPHRITIS. N. Wasserstrum, D.E. Patton Baylor College of Medicine, Dept OB/GYN, Houston, Texas.

We sought to determine if the marked suppresion of fetal breathing movements (FBM) and fetal body movements (FM) we previously reported during fever in pyelonephritis (PYL) depended on etiology. Gravida (GA=30+/-4wk) with viral syndromes (VS), were studied at least 24 hr. off antipyretics during fever T=102.9+/-0.8 C) and early convalescence (T=97.7+/-0.5 C) comparable to PYL.

	Temperature	% Time FBM	Total FM in 30 min
Fever	102.9+/-0.8	6.3+/-5.3	9.6+/-5.5
Convalescence	97.7+/-0.5	5.2+/-4.2	24.4+/-10.2
Control	97.3+/-0.3	36.6+/-8.4	19.5+/-2.2

1. In VS, FBM were depressed during fever, but much less severely and with greater variability [range: 0-15%] than in PYL [FBM = 0.9+/-1.1%; range: 0-3%] 2. In VS unlike PYL, FBM depression showed no recovery in convalescence. 3. In contrast, FM in VS were depressed during fever and recovered during convalescence as in PYL. 4. The fetal biophysical effects of febrile illness reflect more than fever per se, and depend on etiology.

392 UMBILICAL CORD BLOOD GAS SAMPLING AND AIR CONTAMINATION

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Umbilical cord blood gas sampling at the time of delivery utilizing a double clamped closed loop of cord is becoming more of a routine procedure. The effect of air contamination on the accuracy of the sample results was studied in 21 patients. Utilizing 1 cc and 3 cc heparinized syringes, 0.5 cc of cord venous blood was obtained anaerobically, then 0.5 cc of air was drawn in and either removed or retained with the sample. Results: 1) In all samples, there was no difference in the pH, pCO_2, HCO_3 or BE. 2) pO_2 was elevated by air contamination only in the 3 cc syringe with 0.5 of retained air ($p<0.05$). No effect was seen in the 1 cc syringe. 3) Time from delivery until the results were obtained did not effect the results nor did the time from obtaining the first sample results to the last sample results. In a separate experiment with 15 patients using the 3 cc syringe, 0.5 cc of venous cord blood was contaminated with 0.1 cc, 0.2 cc, 0.3 cc, 0.4 cc and 0.5 cc of air. Significant increase in pO_2 occurred when greater than 0.2 cc of air was retained. pCO_2 appeared to decrease while pH and HCO_3 were stable with the increase in pO_2 with the other components being essentially unchanged, neonatal diagnosed and care will not be effected by the air contamination. RECOMMENDATION: Umbilical cord blood obtained for gas analysis at delivery would be best performed with either a 1 cc or 3 cc syringe. If a 3 cc syringe is used, less than 20% of retained air should contaminate the system so as not to confuse the actual pO_2 determination.

393 FETAL BLOOD SAMPLING IN HIV-SEROPOSITIVE PREGNANCIES PRIOR TO ELECTIVE TERMINATION OF PREGNANCY.

RR Viscarello, MT Cullen, NJ DeGennaro*, and JC Hobbins, Depts. of OB/GYN, Yale University, New Haven, CT. and University of South Florida, Jacksonville, FL.

Currently there is no specific immunologic, virologic, or serologic marker for the prenatal diagnosis of HIV-1 infection. We studied 18 HIV-seropositive women between 9 and 24 weeks of gestation prior to elective termination of pregnancy to investigate the transplacental transfer of HIV-1 antibody and p24 antigen and to explore the diagnostic potential of fetal blood sampling in the prenatal diagnosis of intrauterine HIV-1 infection. There were 7 Blacks, 6 Caucasians, and 5 Hispanics with a mean age of 27.2 years. Twelve of the women were CDC Group II, 5 were Group III, and 1 patient had AIDS (Group IV). Eleven patients acquired HIV-infection through IVDA, 6 via heterosexual contact, and 1 patient had no known risk factor. Fetal blood was obtained transabdominally via a single insertion into an anterior cord in 15 cases and transcervically using an embryoscope in 3 patients. HIV-1 antibody was detected by Western blot analysis in all samples of maternal serum, amniotic fluid, and fetal serum. Each mother/fetus pair displayed identical banding patterns. In contrast, p24 antigen was found in the maternal serum and amniotic fluid from only 5 of 18 patients, 3 of which also had p24 in the fetal serum. The finding that 2 of 5 fetal serum samples lacked p24 antigen argues against immediate procedure-related contamination of the fetal compartment. HIV p24 antigen was only found in fetuses of patients with CDC Stage III disease. Of note, p24 antigen was not detected in the 3 fetuses sampled in the first-trimester. Although the risk of iatrogenic infection of the fetus remains to be determined, we conclude that fetal blood sampling in conjunction with a positive p24 antigen result has the potential to provide the diagnosis in utero. (This research was partially supported by a grant from the American Foundation for AIDS Research and the Pediatric AIDS Foundation AmFAR/PAF #50034-7).

394 DOES AN ANTEPARTUM INVASIVE PROCEDURE INCREASE THE RISK OF PERINATAL TRANSMISSION OF HIV-1? RR Viscarello, NJ DeGennaro*, SM Griffith*, W Andiman*, JC Hobbins, Department of OB/GYN, Yale University School of Medicine, New Haven, CT.

Accurate prenatal diagnosis would facilitate more effective patient counseling regarding the risk of vertical transmission of HIV-1. However, the infectious potential of invasive diagnostic methods remains controversial. To determine if the risk of HIV transmission is increased after antepartum invasive procedures, we compared neonatal outcome in 2 groups. Group I consisted of 46 pregnancies in which amniocenteses, fetal blood samplings, fetal scalp samplings, or fetal scalp electrode placements were performed. There were a total of 32 amniocenteses (PTL-18; PROM-9; L/S & PG-5); 18 fetal scalp electrode placements; 10 fetal scalp samplings; and 3 fetal blood samplings (ITP-2; fetal distress-1). Group II included 93 pregnancies without invasive procedures. Both groups were matched for maternal age (27.0 vs. 27.6 yrs), HIV-status, racial breakdown, and HIV-risk behavior. The groups did not differ with respect to CD4 count (401 vs. 385/cc), HIV p24 antigen status, gestational age at delivery (36.2 vs. 36.4 wks), or birthweight (2426 vs. 2754g). No statistically significant difference was noted between the groups with respect to infant disease status with all invasive procedures (chi sq = 0.99), or with amniocentesis alone (chi sq = 1.37). Infant outcome did not differ significantly based on the number of invasive procedures performed during pregnancy (chi sq = 2.26). The mean time interval from invasive procedure to delivery was the same in infected infants and those who seroreverted (t = 0.49). Our data suggests that modern techniques of invasive prenatal diagnosis may predict which fetuses are truly infected with HIV-1 without increasing the risk of iatrogenic infection. (This research was partially supported by a grant from the American Foundation for AIDS Research and the Pediatric AIDS Foundation AmFAR/PAF #50034-7).

395 THE PREVALENCE AND PROGNOSTIC SIGNIFICANCE OF ANTICARDIOLIPIN ANTIBODIES IN PREGNANCIES COMPLICATED BY HIV-1 INFECTION.

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Anticardiolipin antibodies (ACA) are estimated to occur in 2.2% of all pregnancies and are associated with adverse outcomes including thrombotic events, fetal wastage, IUGR, and preterm delivery. Recent studies suggest that maternal antibodies against specific epitopes of HIV-1 may prevent transmission to the fetus. We tested 23 HIV-positive gravidae for ACA to investigate the association with pregnancy outcome, disease status, and perinatal transmission. The racial breakdown included 16 Blacks, 5 Hispanics, and 2 Caucasians. Seventeen patients were IVDAs and 6 were heterosexual partners of IVDAs. Four patients were CDC Group IV; 5-Group III; and 14-Group II; with a mean age of 29.4 yrs. Two of 21 patients (9.5%) were positive for HIV p24 antigen. Thirty-two percent (7/23) were ACA-positive: 5 had IgM and 2 had IgG. All patients who were ACA-positive had CD4 cell counts below 500/cc. Six patients in the ACA-positive group delivered viable infants (mean gestational age 38 weeks and mean birth weight 3068g), and 1 elected TOP. All of the infants are currently CDC Stage P0. Of the 16 patients in the ACA-negative group, 11 delivered viable infants, including 2 sets of twins (mean gestational age 37.5 weeks and mean birth weight 2564g); 2 remain undelivered; and 3 elected TOP. Four infants have seroreverted, 7 are CDC Stage PO, and 2 are lost to follow-up. No peripartum complications were observed in either the ACA-positive or ACA-negative groups. Western blot patterns did not correlate with the presence of ACA and were not predictive of maternal or infant disease status. Of note, 16/20 patients lacked anti-p17 antibody. We conclude that there is a higher prevalence of ACA in HIV-positive patients, which is not associated with adverse perinatal outcome or maternal HIV status. IgM-ACA may recognize a novel epitope of the HIV-virion and therefore prevent perinatal transmission of HIV-1.

396 **PERINATAL EFFECTS OF *GARDNERELLA VAGINALIS* DECIDUITIS IN THE RABBIT.** N. Field,[x] E. Newton, K. Kagan-Hallet,[x] W. Peairs,[x] Dept. of Ob/Gyn, UTHSC, San Antonio, TX.

Gardnerella vaginalis in conjunction with anaerobic bacteria and genital mycoplasmas is associated with bacterial vaginosis and infection-induced preterm birth. However, minimal attention has been paid to the pathologic role of *Gardnerella vaginalis* alone in preterm labor and perinatal morbidity. We studied the effects of intrauterine infection with *Gardnerella vaginalis* on pregnancy outcome and fetal development in the rabbit. Both uterine horns of rabbits at 70% gestation were inoculated hysteroscopically with either 0.2 ml of 10^5 - 10^7 cfu/ml of *Gardnerella vaginalis* or saline. The animals were observed daily for fever, bleeding, or labor. Animals were sacrificed on day 4 or earlier if premature delivery was recognized. Aerobic and anaerobic cultures of blood, decidua, and peritoneal and amniotic fluid were performed. Maternal and fetal histology was examined in a blinded fashion (KK-H) for evidence of infection and/or injury. Severe brain injury was defined as ≥15% neuronal necrosis. Decidual and amniotic fluid cultures were positive for *Gardnerella vaginalis* in all of the study group animals.

Organism (n)	Fever	Preterm labor	Live births (%)	Fetal wt. (gm±SD)	Placental (gm±SD)	Brain injury (%)
Gardnerella vaginalis (17)	3	2	86/108 (80)*	12.8±3.3†	6.2±1.4†	60*
Placebo (14)	0	0	80/84 (95)	16.8±3.1	8.3±2.0	0

*p<0.03; †p<0.001

Gardnerella vaginalis deciduitis did not uniformly result in maternal illness and/or preterm labor. However, intrauterine infection with *Gardnerella vaginalis* had significant detrimental fetal effects, including death, growth retardation, decreased placental weight, and brain injury.

397 **SHOULD AN AMNIOCENTESIS BE PERFORMED BEFORE A CERCLAGE OPERATION IN PATIENTS PRESENTING WITH CERVICAL DILATATION IN THE MIDTRIMESTER OF PREGNANCY?** R. Romero, R. Gonzalez,[x] W. Sepulveda,[x] F. Brandt,[x] M. Ramirez,[x] M. Mazor, Depts. of Ob/Gyn, Yale Univ. School of Medicine, New Haven, CT; Wayne State Univ., Detroit, MI

Cervical cerclage is a management option for patients presenting with cervical dilatation in the midtrimester of pregnancy. Post-cerclage rupture of membranes and clinical chorioamnionitis are common complications often attributed to this procedure. However, these complications may result from a pre-existing ascending intraamniotic infection rather than from the procedure per se. This study was designed to determine whether microbial invasion of the amniotic cavity is present in patients presenting with cervical dilatation and effacement in the midtrimester of pregnancy. Materials and Methods: Amniocentesis was offered to patients presenting with cervical dilatation (>2 cm) in the midtrimester of pregnancy (gestational age ≤24 weeks) (n = 22). Amniotic fluid was cultured for aerobic, anaerobic bacteria and Mycoplasmas. Results: The prevalence of positive amniotic fluid cultures for microorganisms in women presenting with cervical dilatation and effacement was 59% (13/22). The most frequent isolates were Ureaplasma urealyticum (n = 3), Gardnerella vaginalis (n = 2) and Mycoplasma hominis. All patients who had an intraamniotic infection not detected by Gram stain and who underwent a cerclage operation had serious complications (rupture of membranes, chorioamnionitis or subsequent preterm delivery/abortion). Conclusions: 1) Microbial invasion of the amniotic cavity is present in 59% of patients presenting with cervical dilatation in the midtrimester. 2) Patients who had a cervical cerclage in the presence of microbial invasion ruptured their membranes, developed clinical signs of chorioamnionitis or delivered a preterm neonate. 3) Amniocentesis for microbiologic evaluation should be considered before performing a cerclage operation in patients presenting with cervical dilatation in the midtrimester.

398 **INTERLEUKIN-8; ASSOCIATION WITH AMNIOTIC FLUID LEUKOTAXIS AND CHORIOAMNIONITIS.** Peter H. Cherouny, Glenn A. Pankuch[X], Roberto Romero, John J. Botti, Peter C. Appelbaum[Y]. The Depts. of OB/GYN and Pathology, University Hospital, Penn State University, Hershey, PA and Dept of OB/GYN, Yale University School of Med, New Haven, CT. Supported by March of Dimes Research Grant #6-548

Interleukin-8 (IL-8) is a leukoattractant cytokine that has been shown to be elevated in amniotic fluid (AF) of patients with intraamniotic infection, defined by a positive AF culture. IL-8 also produces neutrophil leukotaxis in the AF-leukotaxis assay (AF LeuA), previously shown as a more accurate marker for histologic chorioamnionitis than AF culture. The purpose of this study was to determine if IL-8 is present in AF in which leukoattractants are detectable by the AF LeuA. AF collected from 32 patients within 48 hours of delivery was evaluated by AF culture, AF LeuA, and immunoassay for IL-8. Placentae were evaluated for histologic chorioamnionitis. 14 of 15 AFs negative for AF LeuA, culture and histology had IL-8 levels below the detection threshold of 1 ng/ml. All patients with detectable leukoattractants had histologic chorioamnionitis and detectable IL-8 and are grouped according to culture results. Conclusions: 1) IL-8 is present in AF with positive leukotaxis and, 2) IL-8 concentration increases significantly with extension of the tissue infection into the amniotic fluid. These data support the hypothesis that detection of amniotic fluid leukoattractants, including IL-8, identifies intrauterine infection before there are detectable amniotic fluid bacteria.

Group 1 (n=8)	Group 2 (n=9)
AF LeuA (+)	AF Leu (+)
AF culture(-)	AF culture(+)
Histology (+)	Histology (+)
IL-8 (18.5,2-36)*	IL-8(71.5,3-244)*

*p<0.05 (IL-8 ng/ml; median, range)

399 **THE IMPACT OF AMNIOINFUSION ON MATERNAL AND NEONATAL MORBIDITY IN PREGNANCIES COMPLICATED BY PRETERM PREMATURE RUPTURE OF MEMBRANES AND AMNIONITIS.**

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University of California, Irvine and Memorial Medical Center of Long Beach.

We evaluated 65 pregnancies complicated by both preterm premature rupture of membranes (PPROM) and amnionitis between 24 and 34 weeks of gestation. All patients were induced after the clinical diagnosis of amnionitis was made. Twenty-seven patients received i.v. antibiotics followed by prophylactic amnioinfusion (AI group); 38 patients received i.v. antibiotics only (non-AI group). Both groups were comparable for gestational age at ROM and at delivery, AFI on admission, latency periods from ROM until amnionitis developed, the time from the diagnosis of amnionitis to delivery and birthweight. In addition, there were similar incidences of variable decelerations and meconium staining in both groups. Only 1 of 27 patients in the AI group developed postpartum endometritis as compared to 18 of 38 patients in the non-AI group (P = 0.0003). The marked difference in the incidence of endometritis between the 2 groups was present regardless of the route of delivery (Vag. del. P = 0.018); (C/S P = 0.038). Delivery by C/S was necessary in only 5 of 27 in the AI group and 19 of 38 patients in the non-AI group (P = 0.0058). Although there was a trend towards an increased incidence of C/S for fetal distress in the non-AI group, this was not found to be statistically significant (P=0.079). No significant differences in perinatal depression/acidosis and neonatal sepsis were found between the 2 groups.

Conclusion: In pregnancies complicated by both PPROM and amnionitis, prophylactic amnioinfusion may significantly decrease maternal morbidity by limiting postpartum infections and the cesarean section rate. This study does not demonstrate improved neonatal outcome with amnioinfusion.

400 **PRENATAL DIAGNOSIS OF HIV INFECTION: THE USE OF CORDOCENTESIS, POLYMERASE CHAIN REACTION, AND P24 ANTIGEN ASSAY**

Mark T Cullen MD, Richard R Viscarello MD, Sharon Paryani MD*, L Sanchez-Ramos M.D, University of Florida Health Science Center, Jacksonville, FL. and Yale University School of Medicine, New Haven, CT.

Perinatal transmission of the Human Immunodeficiency Virus is thought to be between 25 to 30%. Neonatal infection is devastating with a 50% mortality in the first year. Antenatal knowledge of fetal infection would allow the option of pregnancy termination or fetal treatment with antiretroviral agents. Thirteen HIV-infected, pregnant women underwent cordocentesis for clinical indications. Two had AIDS (18%) and 11 were seropositive asymptomatic. A single insertion into an anterior cord was performed in all cases. Blood was sent for HCT, PCR, p24 antigen, T-cell phenotyping, and a Kleihauer-Betke. Gestational age ranged from 22 to 34 weeks (mean of 28.2 ± 5.4). Fetal CD4 counts were available in nine cases. There was no difference between the infected and uninfected groups. There was 1 fetal demise secondary to an abruption at term in a PCR-negative fetus. Three cordocentesis samples were positive for HIV-1 by PCR, 2 were indeterminate, and 7 negative. Of the three fetuses who had positive tests by PCR, 2 have developed AIDS, and one is asymptomatic and <15 months (P0). Both of these fetuses also had negative p24 antigen tests. One false negative PCR test was observed. **Conclusion**: HIV detected by PCR at cordocentesis appears to predict which fetus will develop HIV infection. A negative test is reassuring, but not absolute.

401 **HIV IN PREGNANCY: FACTORS PREDICTIVE OF MATERNAL AND FETAL OUTCOME:**

Mark T. Cullen MD, Issac Delke MD, Joseph Greenhaw MD*, Richard R. Viscarello MD, Sharon Paryani MD*, Luis Sanchez-Ramos MD, University of Florida, Jacksonville, FL. Yale University, New Haven, CT

In a 3 year study period, 7,596 pregnant women were screened for HIV-1. 82 (1.07%) were found to be HIV-seropositive and followed prospectively. The mean age at entry was 24.7 yrs. 23 were IVDAs, 10 admitted to prostitution; 25 had multiple heterosexual partners, 2 had received infected blood products, and 28 had no known risk factors. 71 were asymptomatic HIV-positive, 7 had AIDS, and 1 had ARC. 12 of 82 (14%) chose termination of pregnancy for the following reasons: 2 had hysterectomy for CIN3, 1 had new onset PCP pneumonia, and the rest were elective. To date, 64 deliveries have occurred. There were 56 vaginal deliveries and 8 cesarean sections. There was 1 third trimester stillbirth secondary to an abruption. 2 maternal deaths occurred in the peripartum period, which were HIV-related. 8 mothers experienced infectious morbidity. Mean CD4 nadir, while lower for this group did not reach statistical significance (314 vs 550 p=.06). Of the 63 infants, 13 are infected, 15 are seronegative, and 35 are P0. The perinatal transmission rate is currently 22.4%. Mean birthweight was 2,739g (936-4225) and gestation age was 37.7 weeks. 20% were premature deliveries. There were no differences in demographics, mode of delivery, length of ROM, or the use of a scalp clip between the infected and uninfected groups. Transmission rates were influenced by clinical status in the mother. Eight mothers were placed on AZT for CD4 counts<500/cc. There were no ill effects of the drug on either mother or fetus. **Conclusion:** Clinical status appears to be the best predictor of transmission and pregnancy course.

402 VIRULENCE FACTORS OF PYELONEPHRITIS ASSOCIATED E. COLI FROM PREGNANT WOMEN.

M. Martens*, A. Hart*, B. Nowicki*, S. Nowicki*, C. Peyton*, L. Schailer*, G. Anderson, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas

Pyelonephritis is a serious complication in pregnancy. E. coli is the most frequent organism associated with pyelonephritis. Virulence determinants such as P fimbriae have been determined to be associated with the pathogenesis of pyelonephritis in non-pregnant women. However, virulence factors have not been extensively characterized in pregnant women with pyelonephritis. Different fimbrial types on E. coli including P, Dr and type 1 were detected on E. coli isolates from 15 pregnant and 12 non-pregnant patients with pyelonephritis and 31 non infected individuals. E. coli strains were tested for the presence of P, Dr, and type 1 fimbriae (colonization factors) by agglutination with human pp, Dr (a-) erythrocytes or yeast cells (Table 1).

No./Patient	No. of E. coli	FIMBRIAE TYPE 1	P	Dr	p value*
Pregnant (15) <.002* with pyelonephritis	16	3 (19%)*	12 (75%)*	3 (19%)*	
Non-pregnant(12) <.01* with pyelonephritis	11	5 (45%)	9 (81%)*	3 (27%)*	
Healthy women NS	15	9 (60%)	8 (53%)	4 (27%)	

The results indicate that P fimbriae are expressed with higher frequency in pyelonenephritis - associated E. coli. Among cervical isolates of healthy non-infected fimbrial types are more distributed among all strains with type 1 fimbriae being the most frequent. Therefore, it appears that P fimbriae may be virulence factors associated with the development of pyelonephritis with the greatest risk associated with infection in pregnancy.

403 HORMONAL RECEPTORS AND ADHERENCE OF PYELONEPHRITIS ASSOCIATED ESCHERICHIA COLI IN PREGNANCY.

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The incidence of pyelonephritis is increased in pregnancy, and has been attributed to various alterations of the human host. However an investigation of the changes which pregnancy has on bacteria has not been undertaken. Kinetics of various hormones in pregnancy and incidence of pyelonephritis was analyzed, and a correlation was noted for HCG. HCG and a control growth hormone (GH), were tested for possible receptor binding sites on pathogenic E. coli strains. GH or HCG were coated onto microtitre trays. E. coli, (type 1 pili positive and negative), and guinea pig RBC were added. Erythrocytes not trapped by bacteria-hormone complexes were removed by washing, and 0.001% Tryton X was added to lyse bound red cells. The amount of released hemoglobin was measured in an ELISA reader. Results indicate that pathogenic type 1 fimbriated *E. coli* bind to HCG in a dose dependent fashion. Saturation of *E. coli*-HCG binding occur at 4 ug (approximately 65,000 mIU) of HCG per well. Attachment of *E. coli* cells to HCG was inhibited by alpha- methyl-mannose, which is an inhibitor and receptor analog for type 1 pili adhesin. The alpha subunit of HCG, but not beta or GH, demonstrated dose dependent binding of *E. coli*. We conclude that E. coli via type 1 pili can bind to the mannose receptor on the alpha subunit of HCG. This phenomenon may be important in the pathogenesis of pyelonephritis in the early trimesters of pregnancy due to an increase in adherence to receptors found in genito-urinary tract tissues.

404 SUSCEPTABILITY PATTERNS OF RECENT PYELONEPHRITIS PREGANANCY ASSOCIATED PYELONERITIS BACTERIAL ISOLATES. Mark G. Martens, M.D.*, Rajender Syal, M.D.*, G. D. Anderson, M.D. Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas 77550-2778

The incidence of pyelonephritis is increased in pregnancy and is directly related to the incidence of bacteriuria by pathogenic organisms. Treatment for pyelonephritis in pregnancy has changed over the years secondary to an alteration in the susceptibility patterns of the responsible uropathogen. Ampicillin has generally been replaced by first generation cephalosporins, such as cefazolin, due to the increased rate of ampicillin resistant Enterobacteriaceae, including *E. coli*. The current investigation identified the species and sensitivity pattern of recent pyelonephritis in pregnancy isolates. 129 patients and their microbiologic results were reviewed from 1988-91 with 136 organism recovered including *E. coli* (62%), Klebsiella pneumoniae (8%), Enterococcus (5%), Proteus mirabilis (4%), and Group B streptococci (3%). Susceptibility patterns to ampicillin, first generation cephalosporins (cefazolin), and advanced generation cephalosporins (cefotaxime) demonstrated a continued increase in resistance to beta-lactamase sensitive antibiotics.

		% Resistance		
Organism	n=	Ampicillin	Cefazole	Cefotaxime
E. coli	85	35 (41%)	6 (7%)	0
K. pneumonia	11	11 (100%)	1 (9%)	0
Enterococcus	7	0	7 (100%)	7 (100%)
Enterobacter	2	2 (100%)	2 (100%)	0
Total	105	48 (47%)	16 (15%)	7 (7%)

The organisms were significantly more resistant to ampicillin and cefazolin as compared to cefotaxime ($p < .001$, and $p < .01$ respectively). Clinically efficacy correlated with bacteriologic results. Therefore, it appears the resistance patterns affecting the effectiveness of ampicillin in the treatment of pyelonephritis in pregnancy have spread to the first generation cephalosporins, perhaps requiring the consideration of advanced generation cephalosporins such as cefotaxime as the initial empiric antibiotic of choice.

405 ACCEPTANCE OF HIV TESTING WITH AN ON-SITE PRENATAL CLINIC COUNSELOR. Neil S.Silverman, M.D., Susan M. Weiner, R.N.C., M.S.N.[X], Donna Weist, B.A.[X], Depts. of Ob/Gyn and Pediatrics, Jefferson Medical College, Philadelphia, PA

Background: Heterosexual transmission and drug-related acquisition of human immunodeficiency virus (HIV) have been recognized as major contributors to the rising rate of HIV infection in women of reproductive age. Pregnancy in these women is complicated by the potential for maternal-fetal transmission of the virus. This factor has created social and ethical dilemmas regarding HIV testing in this population, since it may not include appropriate explanation of the implications of HIV infection prior to testing. Objective: This study evaluated patient acceptance of confidential HIV testing in an inner-city prenatal clinic after the placement of an on-site HIV counselor. Methods: All patients registering for prenatal care received HIV counseling, after which they were offered confidential HIV testing. Those patients who consented to testing received a time for post-test counseling and release of results. The number of patients tested was compared to the number of HIV tests ordered in the prior six months via physician-consented, risk-directed testing at prenatal intake. Results:

	Pre-counselor	With Counselor
Pts counselled	814	839
Pts tested (% of counselled)	83 (10.2%)	549 (65%)
Positive results (% of tested)	0	3 (0.4%)
Post-test counselled (% of tested)	N/A	281 (34%)

Conclusions: Confidential HIV testing was well accepted in an inner-city pregnant population in conjunction with a full-time, on-site HIV counselor, with six times as many patients consenting to testing than before the current system was in place. This may result from both improved quality of education as well as separation of HIV testing as a medical directive.

406 HEPATITIS C VIRUS (HCV) IN PREGNANCY: SEROPREVALENCE AND RISK FACTORS FOR INFECTION. Neil S. Silverman, M.D., Brenda Jenkin, M.D.[X], Christine Wu, M.D.[X], Patricia McGillen, M.T. (ASCP)[X], Gerald Knee, M.S.[X], Jefferson Medical College, Phila., PA

Background: Though limited reports do exist describing the prevalence of specific anti-HCV antibodies in post-transfusion and other at-risk populations, none exist in prenatal populations. In order to address the vertical transmissibility of HCV, the prevalence of seropositivity in the population in question must first be determined. The Intents of this study were to: 1) anonymously compare the seroprevalence of anti-HCV in an inner-city prenatal clinic to that of a group of private patients; 2) to assess the use of risk factors to predict an increased rate of anti-HCV seropositivity; 3) to identify co-infection risks for HCV and other blood-borne agents. Methods: Blood samples were collected at the time of routine prenatal screening. They were then anonymously analyzed for HCV, HIV, HTLV-1 and RPR using commercially available assays. Results:

	Overall (n=599)	Clinic (n=444)	Private (n=155)
HCV+	26 (4.3%)	23 (5.2%)	3 (1.5%)
HTLV-1+	5 (0.8%)	4 (0.9%)	1 (0.6%)
HIV+	3 (0.5%)	3 (0.7%)	0 (0%)
HBsAg+	5 (0.8%)	4 (0.9%)	1 (0.6%)
RPR+	16 (2.7%)	14 (3.2%)	2 (1.3%)

Seropositivity for anti-HCV was significantly higher overall and in the clinic than for either HTLV-1 or HIV ($p<0.0001$), a statistic which did not hold for the private patients tested. In the clinic, only 52% of HCV-positive patients would have been predicted via targeting by risk factors; only 1 of 3 positive patients in the private offices would have been so identified. Conclusions: HCV infection may be of greater concern than previously thought in inner-city pregnant populations and, as with hepatitis B, not well-predicted by risk-factor identification. Further study is warranted to determine maternal-fetal transmissibility.

407 PENTAMIDINE PROPHYLAXIS IN PREGNANCY. D.Nanda[X], I Tannenbaum[X], S Landesman[X], H Mendez[X], G Moroso[X], H Minkoff. SUNY/Health science Center, Brooklyn NY. Pneumocystitis carini pneumonia (PCP) is one of the commonest opportunistic Infections (OI) among patients with AIDS. Prophylactic agents such as Aerosolized Pentamidine have substantially reduced the incidence of PCP among susceptible patients. At SUNY/HSCB and KCHC, a study was undertaken to assess the effects of Pentamidine use during pregnancy.

METHOD: Fifteen HIV infected patients with T4 counts <200/cmm or <20% of total lymphocytes were given PCP prophylaxis during pregnancy. Detailed history including that of STDs, drug use and other concomitant medications was taken & complete examination and full laboratory assessment was done on each patient. The patients were then administered 300 mg of Pentamidine via a respiragard nebulizer monthly, during II & III trimesters. Serum Pentamidine levels were drawn within an hour of inhalation & were frozen for HPLC assay. The patients were followed prospectively at prenatal clinics and delivered at the two study institutions.

RESULTS: Fifteen mothers received a total of 35 doses of pentamidine for an average of 2.3 doses per patient (range 1-5). One patient with a history of asthma received 60 mg bimonthly. Five patients received AZT concurrently. None of the patients reported any adverse effects of therapy or breakthrough PCP. Serum levels ranged from undetectable to 4.7ng/ml (reports among non pregnant patients have ranged from 0 to 30.91 ng/ml) . One patient discontinued prophylaxis and developed PCP about 70 days after the last dose. All patients delivered at term; All but one delivered vaginally. Mean birth weight was 3159.07± 380 gms (range 2500 - 3570) and head circumference was 33.9±1.1 (range 31-35mm). Average one and 5 minute apgar scores were 7.9± 1.3 and 8.7± 1.1 respectively. None of the babies developed any immediate problems in the neonatal period.

CONCLUSIONS: 1. Aerosolized pentamidine during pregnancy did not achieve high blood levels during pregnancy. 2. No breakthrough PCP was observed in any patient receiving prophylaxis. 3.Pregnant patients tolerated the drug well without any major side effects. 4. No significant effects on the course of pregnancy were observed. 5. Larger studies are needed to fully determine the efficacy and safety of pentamidine during pregnancy.

408 THE UTILITY OF LUMBAR PUNCTURE IN THE EVALUATION AND TREATMENT OF SYPHILIS. Brown,G*,LaSala AP,Anderson R*,Tose D*.Dept. of Ob/Gyn,Columbia University, New York, NY

Current recommendations for the evaluation of late latent syphilis or disease of unknown duration include cerebrospinal fluid (CSF) examination to rule out neurosyphilis. This assessment requires lumbar puncture (LP) which has the potential morbidity of meningitis and spinal headache. We examined the use of LP in a population of young pregnant women living in New York City. An evaluation of obstetrical patients with a positive rapid plasma reagin (RPR) screening test for syphilis was performed. Between Jan. 1, 1989 and Aug. 23, 1991, 253 of these women were followed at our center. They were evaluated for length of disease and given the appropriate therapy based on CDC guidelines. Twenty-three patients had disease present for less than 1 year and therefore did not require CSF examination. Two hundred-thirty women had disease of unknown duration or for greater than 1 year and were offered LP. Of this group, 12 tested positive for HIV. LP was performed in 70 women (30.4%), including 8 of the HIV positive group. One hundred fifty-five (67.4%) women refused LP, and 2 had unsuccessful taps. Four women came to the emergency room for treatment of spinal headache and several others reported milder headache. Three women had CSF that was positive for syphilis. Each of these patients was also HIV positive. The patients with negative CSF and those who declined LP were treated for presumed late latent syphilis. All of the women were followed with monthly RPR tests and showed the decline in values consistent with successful treatment. It is our conclusion that the potential morbidity of LP outweighs its' benefit in the routine evaluation of our young pregnant population with syphilis. All patients with syphilis should be offered HIV testing. CSF examination should be used for women with the additional risk factor of HIV infection which may hasten the progress of the disease. All treated women with syphilis should be followed with monthly RPR tests. Any evidence of persistent disease despite appropriate therapy may also warrant CSF examination.

409 FETAL SYPHILIS: CORRELATION OF SONOGRAPHIC FINDINGS AND RABBIT INFECTIVITY TESTING. L. Nathan[X], D.M. Twickler[X], M. Peters[X], P. Sanchez[X], G.D. Wendel, Dept. Ob/Gyn, U. Texas Southwestern Med Center., Dallas, TX

Fetal syphilis is the presumed diagnosis when the sonographic findings of fetal hydrops are found in the presence of maternal syphilis infection. In the absence of fetal hydrops, the diagnosis of fetal infection is problematic. Rabbit infectivity testing (RIT) is a specific, sensitive in vivo technique to identify Treponema pallidum in infected material. Amniocentesis and subsequent RIT to identify Treponema pallidum may be used to confirm amniotic fluid/fetal involvement. We sought to correlate antenatal sonographic findings from syphilitic gravidas with the results of amniotic fluid RIT. Twenty one gravidas with primary, secondary or early latent syphilis at 24 weeks or greater underwent real time sonography, including coronal liver measurements, and amniocentesis. Measurements which exceeded the 95th percentile for gestational age were considered abnormal. Fetal infection was confirmed by development of syphilis in rabbits after intratesticular inoculation of amniotic fluid. Eleven specimens had positive RITs and ten had negative RITS. The two groups were compared regarding sonographic findings:

	Positive RIT N=11	Negative RIT N=10	P
Hepatomegaly	9	2	<.01
Placental Thickening	7	6	NS
Ascites	2	0	NS
Enlarged Abdomen	1	0	NS
Hydrops Fetalis	1	0	NS

Conclusion: In over 50% of cases of maternal syphilis, fetal infection occurs. Sonographic hepatomegaly is significantly associated with amniotic fluid/fetal infection detected by RIT. Sonography, prior to maternal syphilis therapy, may identify these affected fetuses who are at risk for treatment failure.

410 CORRELATION BETWEEN THE PRESENCE OF HIV-1 mRNA IN THIRD TRIMESTER PLACENTA AND NEONATAL INFECTION.

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The purpose of this study was twofold: (1) to determine if 35S-mRNA *in situ* hybridization (ISH) can identify HIV-1 in paraffin embedded third trimester placentas from asymptomatic seropositive patients and (2) to determine if the presence of HIV-1 viral mRNA in placental tissue is associated with neonatal infection. The study population was seropositive from 1 to 36 months before delivery, had no history of opportunistic infection, depletion of CD4+ count or AZT exposure. Placentas from 3 of 9 patients demonstrated positive ISH. One infant is HIV-1 infected at 5 months of age based on clinical and laboratory findings. The other 2 infants have no evidence of HIV-1 infection at 6 months. None of the 6 infants with negative placental ISH have clinical or laboratory evidence of infection at 1 to 7 months after delivery. We conclude that the sensitive technique of 35S-mRNA *in situ* hybridization can successfully identify the presence of HIV-1 in paraffin embedded placental tissue, but the presence of viral mRNA does not consistently predict neonatal infection within the first 6 months of life.

411 PREDICTORS OF CESAREAN WOUND DISRUPTION/INFECTION. E.R. Newton, Dept. of Ob/Gyn, The University of Texas Health Science Center, San Antonio, Texas.

Parity, duration of internal monitoring and duration of ruptured membranes predict intraamniotic infection (IAI) and endometritis (ENDO). We examined the relationship between labor and operative characteristics that predict cesarean wound disruption/infection (WOUND). Six hundred fourteen consecutive cesarean sections were reviewed during the postpartum hospitalization for demographic, obstetric and operative characteristics as predictors of WOUND. WOUND was defined as any intentional or unintentional wound opening in the first 6 weeks postpartum. Thirty-two (5.2%) wound infections occurred and eight (25%) were associated with IAI or ENDO. Stepwise logistic regression was used to control for confounders and to identify the risk factors for WOUND. Possible predictors included concurrent infection, age, parity, labor duration, internal fetal monitoring, vaginal exams, duration of surgery, blood loss, weight, height, prophylactic antibiotics, gestational age, birthweight and diabetes.

Predictor	Incidence	Adjusted Odds Ratio	95th CI
Smoker	8.1%	6.67	1.2 - 2.49
Obesity (≥ 100 Kg)	17%	2.64	1.1 - 8.4

Unlike other obstetric infections, labor characteristics did not influence risk of WOUND. The etiology of wound infections after cesarean section appears to be similar to wound infections after gynecologic surgery.

412 **TREATED INTRAAMNIOTIC INFECTION AND OPERATIVE MORBIDITY.** E.R. Newton, Dept. of Ob/Gyn, The Univ. of Tx. HSC, San Antonio, TX.

Surgical manipulation in an infected field is associated with intraoperative and postoperative complications. We studied whether or not intraamniotic infection (IAI), which was treated intrapartum, was associated with more operative complications than cesarean deliveries in patients without IAI. Treated IAI and operative complications were identified in 614 consecut ve cesareans. Intraamniotic infection (IAI) was diagnosed by an intrapartum temperature ≥37.8° with 2 of 5 signs: maternal or fetal tachycardia, leukocytosis, tender uterus or foul lochia. Intrapartum ampicillin plus gentamicin was standard therapy at diagnosis of IAI. Clindamycin was added after the delivery of the fetus. The area under the fever curve (FEVER AREA) was calculated as the total C° - hours above 37° after delivery.

Complications	IAI (n=91)	No IAI (n=523)
Duration of surgery (min., range)	57 (111)	54 (178)
Estimated blood loss ≥ 1500 cc	11 (12%)*	23 (4%)
Uterine lacerations	4 (4%)*	4 (0.8%)
FEVER AREA (°C-hr, range)	42 (169)	43 (203)**
Wound infection	2 (2%)	32 (6%)
Septic pelvic thrombophlebitis	0	3
Pneumonia or urinary tract infection	0	7
Discharge from hospital ≥8 days	5 (5%)	43 (8%)

*P<0.05, **Endometritis (n=129) only

Cesarean section in the presence of treated IAI does not increase postoperative complications. Increased blood loss may be related to increased trauma and/or uterine atony associated with intramyometrial infecton.

413 **DOES GROUP B STREP(GBS) COLONIZATION SHORTEN THE LATENCY PHASE OF PATIENTS WITH PRETERM PREMATURE RUPTURE OF MEMBRANES (PPROM)?** Towers CV, Lewis DL, Asrat T, Haraguchi K[x], Perlow JH, Memorial Women's Hospital, Long Beach, CA, University of California, Irvine, CA.

A common premise in patients with PPROM is that GBS colonization will shorten the latency time (defined as time of rupture to time of delivery) when compared to patients who are not GBS colonized. Due to recent literature which emphasizes the impact of digital vaginal exam (DVE) on the latency phase in patients with PPROM, we looked at GBS colonization controlling for incidence of DVE. From 1/86 to 6/91, 577 patients with PPROM between 24 and 35 weeks were evaluated. Patients with multiple gestations, cerclage, advanced labor and indicated deliveries (i.e., pulmonary maturity, etc.) were excluded This left 332 patients for analysis. No patients received tocolysis after PPROM. 43 patients were GBS positive and 289 were negative. No differences were found in gravity, parity, gestational age at PPROM. incidence of DVE and antepartum antibiotic usage between the two groups. The latency in days for GBS positive patients was 6.9± 10.6 and for GBS negative patients was 6.5 ± 10.3. Both groups were then subanalyzed, excluding cases with DVE as shown below.

	GBS Positive	GBS Negative	p Value
Number	26	151	
Gravity	3.5±2.4	2.9±1.7	0.12
Parity	1.3±1.4	1.0±1.0	0.19
Gest.Age PPROM	29.2±2.6	29.6±3.0	0.21
Antibiotics	7	21	0.16
Latency (Days)	9.6±12.3	10.4±12.6	0.76

CONCLUSIONS: GBS colonization, by itself, does not appear to affect the latency phase in patients with PPROM. These data further emphasize the significant shortening of the latency period seen in patients with PPROM who experience a digital vaginal exam.

414 ROUTINE SCREENING OF PREGNANT WOMEN FOR LYME DISEASE IN AN ENDEMIC AREA "IS IT WORTH IT". R. Figueroa, U. Verma, M. Agüero[x], C. Smith[x], N. Tejani. NY Med., Coll., Valhalla, N Y.

Objective Screening for Lyme disease (LD) in pregnant women would detect asymptomatic women with the disease. **Study design** The sera of 485 asymptomatic pregnant women, who received prenatal care at our institution, were tested for LD utilizing the ELISA method (Lyme Stat Test Kit). The test was reported as positive (POS), equivocal (EQUIV), or negative (NEG). The sera of the patients who tested POS or EQUIV was further tested by a Western Blot (WB) for confirmation. The WB was reported as positive (pos) or negative (neg) depending on the appearance of specific (41, 34,31,20) IgG and IgM bands. **Results**

			WESTERN BLOT	
ELISA	#	(%)	Pos	Neg
POS	38	(7.8)	1 (2.6)	37 (97.4)
EQUIV	82	(16.9)	3 (3.6)	79 (96.4)
NEG	365	(75.3)	-	-

Ten patients who tested ELISA POS were found to have syphilis. One of these was the only pos WB. **Conclusions** 1) 25% of the patients had an abnormal ELISA. Only 3.3% of these were true positive to LD (See Above). 2) Routine screening by the ELISA test for LD is not productive or cost effective. ($70 per ELISA, $100 per WB).

415 IMPACT OF ASYMPTOMATIC GARDNERELLA VAGINALIS CARRIAGE ON PREGNANCY OUTCOME. Jean Ricci Goodman, Deidre Spelliscy Gifford[x], UCLA School of Medicine, Los Angeles, California.

This prospective study was conducted to determine whether asymptomatic carriage of gardnerella vaginalis, a component of bacterial vaginosis, is associated with an increased risk of premature labor, preterm premature rupture of membranes (PPROM), low birth weight, and/or maternal infectious morbidity. 176 consecutive asymptomatic obstetric patients had cervico-vaginal cultures for gonorrhea, chlamydia, ureaplasma, mycoplasma, group B streptococcus, gardnerella vaginalis, and other aerobic and anaerobic bacteria. The prevalence of asymptomatic gardnerella vaginalis carriage was 26.7% (47/176). All cultures were obtained prior to 24 weeks gestation (mean time of culture 14.00+/-4.00). Of the initial 176 patients, 32 were culture positive for only gardnerella vaginalis (Group A) and 85 were culture negative for all organisms (Group B). A comparison between these two groups revealed no difference in mean birthweight (Group A 3170+/-851 kg; Group B 3308+/-638 kg) or mean gestational age at delivery (Group A 39.01+/-3.44 wks; Group B 39.37+/-3.04 wks). Nor was the incidence of preterm labor, PPROM, amnionitis, endometritis, wound infection or episiotomy infection significantly different between the two groups. We conclude that asymptomatic carriage of only gardnerella vaginalis is not associated with an increased risk of poor pregnancy outcome. Therefore treatment of obstetric patients who are asymptomatic carriers of gardnerella vaginalis is not warranted.

416 ANTIRETROVIRAL THERAPY DURING PREGNANCY AND POSTPARTUM. U. Taylor and A. Bardeguez, Department of Obstetrics/Gynecology, UMDNJ-New Jersey Medical School, Newark, New Jersey.

The number of AIDS cases reported worldwide in women is rapidly increasing. In 1987 AIDS was the 8th leading cause of death for women of reproductive age in the U.S.A. Zidovudine (AZT) therapy in HIV infected individuals is known to prolong survival in patients with AIDS and to delay the progression of disease in those with CD_4 counts <500/mm³. The major toxicity of AZT is myelosuppression. Other minor adverse effects are nausea, headache and transaminase elevation. The available information on the use of AZT in pregnancy has focused on the lack of adverse effects in the fetus. This report details our experience with the use of AZT during pregnancy and postpartum. As of August, 1991, we have managed 20 patients (10 antepartum and 10 postpartum) with antiretroviral therapy. The mean duration of therapy was 14.1 ± 10 weeks. Ninety percent of our patients were black, 5% hispanic and 5% white. Seven patients were former IVDU's and 13 acquired the infection through heterosexual contact. There were 5 patients with AIDS; 6 with ARC and 9 asymptomatic patients. Mean maternal age was 26.1± 5.6 years. The CD_4 counts ranged from 13-500/mm³ with a mean value of 284.45 ± 187.35/mm³. Laboratory data showed mean value of WBC 7.8×10^3 ± 2.7, hemoglobin 8.9 ± 1.6g/dl, hematocrit 27.6% ± 3.2, GOT 35 ± 11.2; and GPT 39 ± 9.1 for that series. The obstetrical complications in the series were: preterm labor (3), PROM (1) and chorioamnionitis (1). Nineteen patients have delivered grossly normal infants with mean birth weight of 2299g. No fetal anomalies were noted. In patients with antepartum treatment, transaminase values were unchanged and CD_4 counts show an upward trend. We conclude that AZT has no immediate adverse effect on the pregnant woman or her neonate. Our findings support the use of AZT in pregnant women who are significantly immunocompromised or have advanced stage disease.

417 HIV-ASSOCIATED IMMUNE THROMBOCYTOPENIA IN PREGNANCY. U. Taylor, P. Gascon, J. Apuzzio and A. Bardeguez. Departments of Obstetrics/Gynecology and Hematology, UMDNJ-New Jersey Medical School, Newark, New Jersey.

Immune thrombocytopenia purpura (ITP) while relatively uncommon during pregnancy (<2%); occurs in 10 to 15% of asymptomatic HIV infected patients and is often the initial manifestation of the disease. Zidovudine (AZT) has been used effectively to treat HIV related thrombocytopenia. We sought to determine the prevalence of ITP in HIV infected pregnant women and to develop appropriate management guidelines for these cases. Between 1986 and 1990, we analyzed all HIV infected pregnant patients in our cohort with platelet counts <100,000/mm³. We evaluated a total of 112 cases and identified nine cases (8%) with thrombocytopenia. The HIV risk factors for these 9 cases with thrombocytopenia were IVDA (4/9) and heterosexual contact (5/9). There was 1 patient with AIDS, 6 with ARC; and 2 others were HIV positive but asymptomatic. The mean maternal age was 30.3 ± 3.9 years and the mean platelet count was 70,000 ± 21,000. Four patients had positive antiplatelet antibody. The CD_4 counts in these patients were 287.7 ± 106.7/mm³. The two patients treated with AZT for an average of 12 weeks during pregnancy, showed an increase in platelet count of 26%. These pregnancies were delivered at term. No IVH or other bleeding diathesis were noted in the neonates. Late postpartum hemorrhage was a complication in 2 patients. Thrombocytopenia was a common manifestation of HIV infection in our series. We recommend the inclusion of HIV testing in the diagnostic evaluation of thrombocytopenia for women of reproductive age. When other diseases have been ruled out intervention with antiretroviral therapy is beneficial to these patients.

418 **QUALITATIVE PHYSICAL AND CHEMICAL CHANGES OF INFECTED AMNIOTIC FLUID - AN IN VITRO STUDY.** W. Scorza, P. Lewis, A. Vintzileos, Mt Sinai Hosp., Univ. of CT Health Ctr, Farmington, CT

It has been a clinical observation that patients with ruptured membranes and subsequent infection fail to demonstrate arborization on the fern test. The purpose of this study was to determine if amniotic fluid inoculated with known pathogens differed in its arborization pattern and if qualitative changes (glucose, protein, nitrites, pH and leukocyte esterase) could be detected using a standard reagent strip. Sterile amniotic fluid was collected from 25 amniocenteses performed in the 2nd or 3rd trimesters. Patients with ruptured membranes, premature labor or signs of intraamniotic infection were excluded. Each specimen was divided into 5 cc aliquots consisting of a control and inoculated samples with either Group B *strep*, *E. coli*, or *B. fragilis*. The investigators who analyzed the amniotic fluid were blinded. The fluid was incubated at 38°C and examined at 48 and 96 hours for Gram stain, culture, and arborization and qualitative changes. Fifteen specimens were inoculated with each organism and compared to controls all of which had negative Gram stains and cultures. All inoculated samples had positive Gram stains and cultures. Inoculated specimens and controls demonstrated similar arborization patterns at 48 and 96 hours. No sample failed to "fern". All inoculated samples revealed "negative" readings for glucose (sensitivity of the reagent, 74 mg%) while controls were trace positive (sensitivity of the reagent, 100 mg%). There were no differences in protein (3+), nitrites (negative), pH (7.5 - 8.0), or leukocyte esterase (negative). The results were the same at 48 and 96 hours. This in vitro study confirms the usefulness of the fern test to diagnose ruptured membranes in the presence of infected amniotic fluid. This study also suggests that development of a reagent strip with high sensitivity for glucose may prove useful in the diagnosis of infected amniotic fluid. The qualitative analysis of other biochemical parameters did not seem to be useful at least in vitro.

419 COMPARISON OF GRAM STAIN, LEUKOCYTE ESTERASE, AND AMNIOTIC FLUID GLUCOSE IN PREDICTING CULTURE RESULTS IN PATIENTS WITH PREMATURE RUPTURE OF MEMBRANES. D. Gauthier,[X] W. Meyer,[X] A. Bieniarz. University of Illinois, Chicago, IL

Gram stain(GS), leukocyte esterase activity (LE), and amniotic fluid glucose(AFG) have been described as rapid predictors of amniotic fluid culture results. METHODS. A prospective study was performed on 90 patients with PROM ≤34 weeks EGA and no clinical evidence of infection. Aerobic, anaerobic, and mycoplasma cultures were done as well as GS, LE, and AFG on fluid obtained by amniocentesis. RESULTS. Cultures were positive in 47 patients(52%). The sensitivity, specificity, positive and negative predictive values of GS, LE 1+ or 2+, and AFG ≤16 mg/dl in predicting culture results are outlined below:

	SENS	SPEC	PPV	NPV
Gram st.	38(49)	95(95)	90(90)	59(68)
LE 1+/2+	68(81)*	84(84)	82(81)	71(83)
AFG ≤16	75(89)*	91(91)	90(89)	77(91)#

()=Exclusion of cultures + for Ureaplasma alone with no evidence of maternal/neonat. infection. In comparison to GS- * = $p < .01$, # = $p < .05$

CONCLUSION: AFG AND LE WERE SIGNIFICANTLY MORE SENSITIVE THAN GS IN DETECTING POSITIVE CULTURE RESULTS. OVERALL, AFG APPEARS TO BE THE MOST ACCURATE RAPID PREDICTOR OF CULTURE RESULTS.

420 FREQUENCY OF UTERINE CONTRACTIONS AFTER ANTIBIOTIC THERAPY FOR PYELONEPHRITIS: Graham JM*, Blanco JD, Oshiro BT, Magee KP: Department of Obstetrics, Gynecology and Reproductive Sciences, LBJ General Hospital, UTHSC-Houston, Texas.

Some pregnant women with acute pyelonephritis (Pyelo) present with uterine contractions (UCs). Presently, the relationship of UCs and antibiotic (AB) administration (with subsequent bacterial disruption) is unclear. We studied the relationship of AB therapy and UCs in pregnant patients with Pyelo. We recorded the number of UCs prior to and after initial AB therapy in 25 women. We compared the mean number of UCs at each hour after AB treatment to the mean number of UCs in the hour prior to AB administration. We also noted the number of patients who had an increase in UCs at each hour after AB initiation over the pre-AB hour. Inclusion criteria were: IUP ≥ 26 weeks gestation and clinical and laboratory evidence for Pyelo. We excluded any patients who had AB in the 7 days prior to admission. Statistical analysis was performed using ANOVA with a $p \leq 0.05$ as significant.

Hour	Mean Number of UCs	P value	Patients with Increased UCs
-1	7.8 ± 5.0		
Antibiotic administration			
+1	13.0 ± 6.7	0.003	21/25 (84.0%)
+2	14.0 ± 7.7	0.001	23/25 (92.0%)
+3	13.3 ± 7.9	0.005	20/25 (80.0%)
+4	13.0 ± 7.6	0.007	18/22 (81.8%)
+5	11.6 ± 6.3	0.029	13/20 (65.0%)

At study entry, 22 of 25 (88%) patients were < 37 weeks. Six of 25 patients (24%) required tocolysis after AB administration. Nineteen patients have delivered and 4 (21.1%) delivered at < 37 weeks gestation. We found a statistically significant increase in UCs over baseline in hours 1 through 5 after AB administration. Further studies are needed to elucidate the reason why UCs increase after AB administration in the pregnant patient with Pyelo.

421 IN VITRO STUDY OF AMNIOTIC FLUID GRAM STAIN: EFFECT OF CENTRIFUGATION. W. Torres,[X] D. Gauthier,[X] W. Meyer,[X] S. Warsof. University of Illinois at Chicago, Chicago, IL.

Amniotic fluid Gram stain (AF GS) has been used to assess for intraamniotic infection. It has been reported that centrifugation does not improve the sensitivity of AF GS. METHODS. AF obtained by amniocentesis from patients with preterm labor or preterm rupture of membranes was pooled. Individual AF samples as well as the pooled sample had negative GS and cultures. Using pure bacterial cultures, a 0.5 McFarland suspension was made and then diluted into the pooled AF to the concentrations outlined below. Concentrations were confirmed by colony counts. Prior to GS, each sample was divided into two portions, with one undergoing centrifugation. The slides were read in a blinded fashion by microbiology technicians.

	AF -NO CENTRIFUG.					AF-CENTRIFUG.				
BACT/CC	0	10^3	10^4	10^5	10^6	0	10^3	10^4	10^5	10^6
GS +	1	2	9	13	15	1	10	12	16	15
GS -	15	14	7	3	1	15	6	3	0	1
X^2 p-value vs no centrifug.							<.01	.14	NS	NS

CONCLUSIONS: CENTRIFUGATION OF AF PRIOR TO GS IMPROVED SENSITIVITY AT LOWER BACTERIAL CONCENTRATIONS. AT CONCENTRATIONS $\geq 10^5$, CENTRIFUGATION DID NOT IMPROVE ACCURACY OF GS.

422 BLOOD CULTURES ARE NOT COST-EFFECTIVE IN THE INITIAL EVALUATION OF POSTPARTUM ENDOMETRITIS. RD Jelsema[X], NB Isada. Divs Mat-Fetal Med and Reprod Genetics, Hutzel Hosp, Wayne St U, Detroit, Mi.

Objective: To assess the clinical impact and cost-effectiveness of blood cultures in the workup of uncomplicated postpartum endometritis. Design: Chart review of patients with a discharge diagnosis of postpartum endometritis. Setting: Public hospital postpartum ward. Patients: All patients with a discharge diagnosis of postpartum endometritis (n=83) in a 6 month period. Results: 67 patients had blood cultures, both aerobic and anaerobic (42 one set; 25 two sets; total 92 sets) obtained before starting antibiotic therapy. Three cultures were positive for Bacteroides fragilis, Streptococcus agalactiae, and Escherichia coli. The positive cultures did not change the therapy for any patient. The observed proportion of those individuals whose therapy was changed by blood culture results was 0%; binomial analysis shows an upper 95% confidence limit of ~4%. Discussion: The routine use of blood cultures in the initial workup of post-partum febrile patients with uncomplicated endometritis has been questioned because of the low specificity, low sensitivity and the high incidence of false-positive results (up to 50% in some series). This practice requires scrutiny because broad-spectrum or multi-agent antibiotic therapy is effective for such polymicrobial infections without blood culture results, with final identification often available only after the patient is considered cured. Furthermore, if the patient has not improved, evaluation is directed toward such diagnoses as wound infection, pelvic abscess or septic pelvic thrombophlebitis. Since results of blood cultures are unlikely to affect therapy (<4%) and their aggregate cost is substantial, we conclude that it is difficult to demonstrate their clinical utility or cost-effectiveness in the initial workup of uncomplicated postpartum endometritis in our patient population.

423 INCREASED AMNIOTIC FLUID TUMOR NECROSIS FACTOR-α & INTERLEUKIN-1B WITH SEVERITY OF CHORIOAMNIONITIS. Glenn A. Pankuch[X], Peter H. Cherouny, Douglas C. Kuhn[X], John J. Botti, Peter C. Appelbaum[X]. The Depts. of OB/GYN and Pathology, Penn State University, Hershey Med Center, Hershey, PA. Supported by March of Dimes Grant #6-548

Tumor necrosis factor-α (TNFα) and Interleukin-1β(IL1β) levels were determined in 28 amniotic fluids (AF) from patients in preterm labor with intact membranes. The purpose was to compare cytokine levels to results from the leukotaxis assay (LeuA) and microbiologic, histologic and clinical tests for chorioamnionitis. Cytokine levels were determined by ELISA. The fluids are grouped below according to LeuA result, presence of AF infection (positive Gram stain or culture) and clinical infection. AFs in groups 1, 2, and 3 were obtained from patients in idiopathic preterm labor. All patients in groups 2, 3, and 4 delivered within 48 hours of amniocentesis and all had histologic chorioamnionitis and detectable leukoattractants. Cytokines were not detected in AFs from group 1 despite significant cervical dilation. Results indicate; 1) TNF-α and IL-1β do not reach detectable levels in all LeuA (+) fluids and, 2) AF levels of IL-1β significantly increase following extension of the ascending infection into the amniotic cavity (Gp2 vs Gp3, $p<0.05$). TNF-α and IL-1β reach their highest levels in the presence of clinical infection (Gp3 vs Gp4, $p<0.01$).

	Gp 1 n=5	Gp 2 n=7	Gp 3 n=11	Gp 4 n=5
AF LeuA	(-)	(+)	(+)	(+)
AF Infection	(-)	(-)	(+)	(+)
Clinical Infx	(-)	(-)	(-)	(+)
TNFα	0[X]	3 (158±82)[*]	10 (394±112)	5 (1251±294)
IL1β	0[X]	6 (510±169)	11 (1141±217)	5 (2084±144)

[X] # of AFs > detection threshold(TNF,20pg/ml;IL1,4pg/ml) [*] mean±SEM

424 **ICON STREP B^R: AN ENZYME IMMUNOASSAY FOR RAPID DETECTION OF GROUP B STREPTOCOCCUS.**

MJ Paidas[x+], DT Manhoff[x], S Kagan[x], G. Bender[x], J Earl[x], JC Dunn[x].
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A sensitive, reliable, and rapid test for detection of group B streptococcus (GBS) is still needed. Therefore, we evaluated an enzyme immunoassay Icon Strep B^R (Hybritech, San Diego, CA) for the detection of GBS in a population of 62 randomly selected pregnant patients presenting to Labor and Delivery at Pennsylvania Hospital. Three swabs from both the cervix and vagina were obtained per patient. One swab was tested with Icon Strep B^R without incubation. A second swab was placed in Lim Broth Enhancement Media^R (BBL, Baltimore, MD) incubated for 4 hrs at 37°C in a CO_2 rich enviroment followed by testing with Icon Strep B^R. The third swab, a control ,was placed in Lim Broth Enhancement Media^R, incubated for 4 hours at 37°C in a CO_2 rich environment and then plated on blood agar and incubated for 24 hours at 37°C in a CO_2 rich environment, and then read by a medical technologist. Colonies suspected of being positive for GBS were verified by a latex agglutination method.

RESULTS The prevalence of GBS by culture was 21.0% (13/62).

		+ Culture	-
Icon Strep B	+	1	0
	-	12	49

		+ Culture	-
Icon Strep B Lim Broth	+	7	0
	-	6	49

	Icon Strep B	Icon Strep B with Lim Broth
Sensitivity	7.7%	53.8%
Specificity	100%	100%
Positive Predictive Value	100%	100%
Negative Predictive Value	80.3%	89.1%

CONCLUSION In this preliminary study, Icon Strep B has poor sensitivity for rapid detection of GBS. However, its sensitivity is substantially improved with a short period of incubation with enhancement medium. More studies are necessary to confirm these results.

425 **THE EFFECT OF UNTREATED SYPHILIS ON THE MATERNAL CHARACTERISTICS OF LABOR AND DELIVERY**

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Brown Univ/Women & Infants Hospital, Providence, RI.

Syphilis is a significant cause of perinatal morbidity and mortality. A retrospective study of 79 women with positive serologic tests for syphilis who delivered between January 1, 1988 and December 31, 1990 was conducted to determine the intrapartum behavior of the fetus exposed to syphilis. Patients were grouped according to treatment status during pregnancy: group I (n=32) - no treatment, group II (n=12) - inadequate treatment, group III (n=25) adequate treatment, group IV (n=10) - appropriate treatment during the pregnancy but reinfected, and group V (n=25) - control population matched for gestational age and registration status. Infants were classified as to the certainty of the diagnosis of congenital syphilis by both Kaufman's criteria and the newer guidelines issued by the CDC in 1988. Diagnostic categories included probable, possible, unlikely (Kaufman) and compatible, unlikely (CDC). Neonatal diagnosis was found to be dependent on maternal treatment status ($p<0.001$). Intrapartum factors examined included: fetal heart rate tracing characteristics, presence or absence of meconium stained amniotic fluid, route of delivery, gestational age, weight at delivery, and Apgar score. Results were analyzed by analysis of variance and test for differences in proportions. There was no significant difference in the incidence of positive urine toxicology screens between study and control populations. Infants of untreated mothers, and those in the "probable" diagnostic category, had significantly fewer fetal heart rate accelerations, more late decelerations, more deliveries by cesarean section, more preterm deliveries and low birth weight infants. Infants in the "probable" category of congenital syphilis were also more likely to have meconium stained amniotic fluid. The fetal heart rate tracings of treated patients, and those "unlikely" to have congenital syphilis, did not show loss of reactivity or late decelerations. This suggests that maternal treatment of syphilis during pregnancy is beneficial and can prevent poor fetal outcome.

426 **HIV-1 INFECTION AMONG ADOLESCENT PARTURIENTS**

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Objective: To define the prevalence of HIV-1 infection and to characterize HIV risk behavior in adolescent parturients.
Methods: We performed a case-control study of 51 HIV-1 infected and 282 seronegative adolescent parturients (Ages 13-20) identified from a prenatal population undergoing routine voluntary HIV-1 antibody screening with self-reported risk behavior.
Results: From 7/87-3/91, we screened 10,794 adolescent parturients of which 51 (4.7 per 1000) were HIV-1 infected. The demographic characteristics of cases and controls were comparable, however 1/3 of cases were age 17 years or less. Significantly more cases than controls reported a history of crack cocaine use 10 (19.6%) vs. 23 (8.2%) (P=.03). Twenty-two (43%) of cases had no apparent risk factors for infection and 17 (33%) were presumed to be infected by heterosexual contact. Thirty-nine (14%) of controls had self identified risk factors for infection.
Conclusions: Adolescent parturients in our center are at risk for HIV-1 infection and should be targeted for HIV education and risk reduction counseling.

427 **HIV IN PREGNANCY: THE SWISS COLLABORATIVE STUDY**

O. Irion[x1], Ch. Rudin[x2], K. Biedermann[x3], F. Béguin[1] and the members of the Collaborative Group. Depts Ob/Gyn University Hospitals GENEVA[1] and ZURICH[3], Children Hospital University BASEL[2], Switzerland

Switzerland has the highest rate of AIDS cases in Europe: 1891 cases (283/million) until june 1991; 13511 HIV+ tests have been anonymously reported to the Federal Office for Public Health. Women represent a third of all cases. Many questions regarding HIV and pregnancy remain unanswered. Asymptomatic HIV infection is not a recognized risk for pregnancy complications. Vertical transmission rate is estimated 10 to 30%. It is not clear whether pregnancy influences HIV progression. Data are lacking on treatments during pregnancy. **Methods:** this multicentric prospective study was designed to collect epidemiological data, to study interactions between HIV and pregnancy, and to identify markers for vertical transmission. Pregnant women are included after informed consent. History, physical examination and laboratory are repeated at 3 months intervals, 1.5 and 6 months post-partum. When possible, women are then followed in the national cohort study. Neonates are included in the swiss neonatal study. **Results:** from may 1st 90 to aug. 20th 91, 96 HIV+ pregnant women were included: 39 (40.6%) obtained an abortion, 57 (59.4%) carried on their pregnancy, 44 have now delivered. We found no differences between women obtaining an abortion and those pursuing pregnancy for mean age (27.2 y, 19-35 vs 27, 21-35), way of infection (IVDU 68.4 vs 70.1%, sexually 28.9 vs 24.5%, p=NS) or CDC stage (II: 54.2 vs 73.6%, III: 37.1 vs 18.9%, IV: 8.6 vs 7.5%, p=NS). Six women seroconverted during pregnancy. In 78.7% of cases the first positive test was known before 1990. Twenty-eight sexual partners were also HIV+, mostly IVDUs. We have data on 40 deliveries of 42 newborns: 20 females, 22 males, 2 sets of twins. Mean gestational age is 37.8 weeks (27-41), mean birthweight 2860 g (900-4200); 23 deliveries (57.5%) were spontaneous, 4 instrumental (10%) , 13 by cesarean section (32.5%: 7 CS (17.5%) were electively performed in one center). **Conclusion:** after 16 months, we collected an unexpectedly high number of patients. Updated data will be presented. The study is ongoing and will help to improve the care of pregnant HIV+ women.

428 **PERINATAL HIV: OUTCOME AND MATERNAL FACTORS RELATING TO TRANSMISSION.** A Sison,[X] J Sever,[X] C Brandt,[X] T Rakusan,[X] M Chan,[X] J Campos,[X] D Fuccillo,[X] E Saxena,[X] M Young.[X] Georgetown, DC General and Children's Hospital, Washington DC.

Through pregnancy and at delivery, 25 HIV infected women and their newborns were followed & tested for HIV & HTLV1 antibody, titer of HIV IgG and T cell subsets. Proviral HIV DNA by polymerase chain reaction (PCR) and virus by culture (VC) were also tested. **RESULTS: [MOTHER]** All but one were asymptomatic. Maternal IgG titers were high (mean=1:3450). CD4 counts were depressed (mean=615). Co-infection with HTLV1(=1) was rare. There was no difference between mothers of infected infants vs. those with indeterminate status regarding IgG titer, time to positivity of VC, or length of seropositivity. **[INFANT]** 20% were born < 36 wks. Known infected infants had mothers who were PCR+ and VC+ at delivery.

	Mother(n=20)	Neonate(n=20) HIV-Infected	Inderminate
PCR+	17/20	3/3	1/17
VC+	10/20	3/3	0/17

CONCLUSIONS: Most infected pregnant women (despite CD4 depression) and almost all infants of infected mothers are asymptomatic at birth. Prematurity appears increased in HIV+ pregnancies. Maternal HIV IgG titers are high but does not seem to protect against viral transmission.

429 THE ASSOCIATION OF CLINICAL INTRAAMNIOTIC INFECTION (IAI) AND MECONIUM. Wen IS*, Eriksen NL*, Graham JM*, Blanco JD, Oshiro BT*, Prieto JA. Dept Obstetrics, Gynecology, and Reproductive Sciences, LBJ Hospital, UTHSC, Houston, Texas.

Meconium has been shown to enhance bacterial growth. However, its role in IAI is undetermined. To determine the rate of IAI in patients with meconium and controls, we compared 100 pregnant women with meconium and 100 pregnant women without meconium between September 1 and December 31, 1990. Exclusion criteria were any active infection prior to labor or antibiotic use within the 7 days prior to delivery. We diagnosed clinical IAI in patients with rupture of membranes (ROM), maternal fever ≥ 100.4°F and any 2 of the following: maternal or fetal tachycardia, uterine tenderness, WBC ≥ 10,500mm^3 or foul amniotic fluid. We analyzed continuous variables by the Wilcoxon rank test and discrete variables by Chi-square or Fisher's Exact test as appropriate. All clinical characteristics are presented as the mean ± standard error.

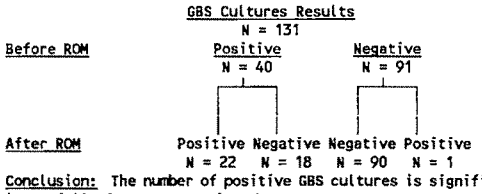
Characteristics	Meconium (N=100)	No Meconium (N=100)	P
Age(yrs)	23.6 ± 0.5	24.1 ± 0.6	NS
Parity	1.1 ± 0.1	1.3 ± 0.1	NS
Length of Labor (hrs)	10.6 ± 0.8	10.5 ± 0.6	NS
Length of ROM (hrs)	5.0 ± 0.8	6.3 ± 1.2	NS
Vaginal exams (#)	4.1 ± 0.2	4.3 ± 0.2	NS

The percentage of patients with an intrauterine pressure catheter was similar between the meconium (17%) and no mecorium (21%) groups (P=NS). The rate of clinical IAI was significantly higher in women with meconium-stained amniotic fluid (8%) compared to women with no meconium (2%) (p=0.05). We conclude that the rate of clinical IAI is higher in patients with meconium-stained fluid compared to patients without meconium.

430 **GROUP B STREPTOCOCCUS CULTURES FOLLOWING RUPTURE OF AMNIOTIC MEMBRANES.** C.E. Henderson, H. Egre[X], G. Szilagyi[X], M.Y. Divon. Dept. of Ob/Gyn, Albert Einstein College of Medicine, Bronx, New York.

Amniotic fluid has been shown to exhibit specific antibacterial activity against group B Streptococcus (GBS). Therefore, genitourinary tract cultures used to identify women colonized with GBS before rupture of membranes (ROM) may have different results after ROM. To evaluate the effect of ROM on a rapid culture technique to identify GBS (sensitivity of 98.4% and specificity of 100%), we performed cultures on 131 women before and after ROM. Rayon tipped swabs were used to obtain samples from the vaginal introitus, the specimen was then incubated in selective broth medium to facilitate rapid bacterial growth. Samples positive for GBS were identified within 12 to 24 hours by slide co-agglutination.

Results: Before rupture of membranes 40/131 (30.5%) were GBS positive. After rupture of membranes only 22 of these 40 cultures (55%) remained positive. In contrast, only 1/91 (1%) culture which was initially negative became positive. The number of positive cultures before rupture of membranes 40/131 was significantly greater than the 23/131 seen after rupture of membranes (p = 0.02).



Conclusion: The number of positive GBS cultures is significantly lower following rupture of membranes.

431 **OUTCOME OF LYME DISEASE IN PREGNANCY.** Jodi F. Abbott, M.D.[X], Neil S. Silverman, M.D. Jefferson Medical College of Thomas Jefferson University Hospital, Philadelphia, PA.

The natural history of Lyme disease in pregnancy is not well understood, though the transplacental transmission of Borrelia burgdorferi has been documented. Case reports of cardiac malformations, fetal and neonatal deaths following maternal exposure have been published, though cause and effect have been difficult to establish. We sought to follow women with positive Lyme serology prospectively through pregnancy to help define fetal risks of in utero exposure. Materials and Methods: All patients were referred for consultation or management due to their history of possible Borrelia exposure. Six women have been followed with a clinical or laboratory diagnosis of Lyme disease. All had negative RPR's, eliminating cross reaction with other spirochetal antibodies.

Pt	Clin Sx	IgG	IgM	Trim Exp	Outcome
1	+	+	-	3rd	NI, term, AGA
2	+	+	-	2nd	NI, term, AGA
3	-	+	+	2nd	NI, term, AGA
4	+	+	-	1st	NI, term, AGA
5	+	+	-	1st	Ongoing
6	+	+	-	1st	Ongoing

Patients 1-3, with early Lyme disease received oral amoxicillin for at least three weeks. Patients 4-5, with systemic disease, received 1 mo. IV ceftriaxone, then ongoing oral amoxicillin. Patient 6 is receiving continuing IV ceftriaxone. All fetuses had normal cardiac ultrasounds in the second trimester. Cord blood was obtained on two newborns; #1 had +IgG -IgM; #2 had -IgG -IgM. Neither patient had evidence of spirochetes in their placentas. Discussion: To date, this is one of the larger prospective reports of fetal outcome in pregnancies exposed to Lyme disease. Although a limited series, the normal outcomes of these patients is reassuring, and may be helpful in patient counselling.

432 PETHIDINE COMPARED WITH DIAMORPHINE FOR PAIN RELIEF IN LABOUR. F.M.Fairlie, L. Marshall[X], J.J. Walker The Perinatal Centre, Glasgow Royal Maternity Hospital, Glasgow, Scotland. U.K.

Despite its proven inefficiency, intramuscular (IM) pethidine is widely selected for pain relief in labour. Diamorphine has been shown to be more effective but there has been a reluctance to use this powerful analgesic in labour. This study was designed to compare the analgesic properties and side effects of IM pethidine with IM diamorphine in active labour. **Method:** 50 nulliparous and 50 multiparous women in active labour (Bishop score ≥5) were randomly assigned to receive either IM pethidine or IM diamorphine. Nulliparous women received either 150 mg pethidine or 7.5mg diamorphine, multiparous women received either 100mg pethidine or 5mg diamorphine. Pain severity was assessed by a visual analogue score and a 5 point verbal scale. Assessments were made immediately before analgesia and at intervals of 30 minutes post analgesia until delivery or until additional analgesia (epidural blockade) was requested. **Results:** For each parity group there was no difference between those receiving pethidine compared with those receiving diamorphine with respect to bishop score at the time of drug administration, duration of labour or subsequent epidural analgesia. For the nulliparous group, pethidine was associated with a significant reduction in pain severity at 30 minute (p=0.03 Wilcoxon signed rank test) and 60 minutes (0.01) post drug administration. Reduction in pain severity was more marked in the diamorphine group (0.001 at 30 minutes and 0.008 at 60 minutes). The need for neonatal resuscitation was significantly less for the diamorphine group (p=0.03). The multiparous group showed similar differences. **Conclusion:** This data suggests that IM diamorphine is associated with less neonatal sedation compared with IM pethidine and there appeared to be a trend towards greater pain relief in the diamorphine group.

433 INDWELLING SPINAL CATHETERS AND POST DURAL PUNCTURE HEADACHE. S. Cohen,[X] N.Singer,[X] D. Amar,[X] M. Divon, Depts. of Anesthesia and Obstetrics/Gynecology, Albert Einstein College of Medicine, Bronx, NY.

Puncture of the dura mater for regional anesthesia is occasionally associated with post dural puncture headache (PDPH). It has been suggested that continuous spinal anesthesia (CSA) is associated with a low incidence of PDPH in non-obstetric patients. The purpose of this study was to determine the incidence of PDPH following inadvertent dural puncture in term pregnant patients and to assess the effect of continuous spinal anesthesia on PDPH. An epidural block (EB) was attempted in 65 patients. Three groups were identified: Group I (n=24) had a dural puncture on the first attempt of EB followed by a successful EB on the second attempt. Group II (n=26) had a dural puncture immediately converted to CSA with the catheter left in situ <24 hours. Group III (n=13) same as Group II, but the catheter left in situ >24 hours. Results:

	PDPH	Duration of Spinal Catheterization (hrs)
Group I	10/24	0
Group II	12/26*	8.3 ± 4.7 **
Group III	0	37 ± 9.7

* p<.01 Group III vs. Group I or II
** p<0.001 Group II vs. Group III.

In conclusion, continuous spinal catheterization following accidental dural puncture may be an adequate method of PDPH prophylaxis in pregnant patients.

434 THE EFFECTS OF CONTINUOUS BUPIVACAINE/FENTANYL EPIDURAL ON FETAL HEART RATE CHARACTERISTICS. E.R. Newton, B. Schroeder,[x] K. Higby[x] and B. Bennett,[x] Dept. of Ob/Gyn, The Univ. of Tx. HSC, San Antonio, Tx.

The effects of epidural analgesia on fetal heart rate patterns (FHR) are controversial. The differences in maternal and fetal conditions between patients obscure the direct effect of epidurals on FHR. We evaluated the effects of epidural analgesia on FHR using each fetus as its own control. Thirty-seven successful continuous labor epidurals using bupivacaine and fentanyl were matched with the next two consecutive patients of the same parity who delivered without epidural analgesia. Fetal heart tracings, one hour before and after epidural placement, or at a corresponding cervical dilation in the control patients were evaluated by in a blinded fashion. Six (16%) epidural patients required ephedrine for hypotension and epidural patients received more I.V. fluids than controls (2075 vs. 952 cc, P <0.01). FHR characteristics within fetuses in the presence or absence of epidural analgesia, were compared.

	Mean change between periods		
Characteristics	Epidural		Control
Baseline heart rate (BPM)	1.4		−1.1
Variability (BPM)	−0.9		−1.7
Maximum oscillation (BPM)	0.32		−1.3
Accelerations	−0.81	p=ns	−1.88
Moderate variables	0.81		0.82
Severe variables	0.00		0.47
Late decelerations	0.20		0.54

Deterioration or improvement in FHR characteristics are not related to continuous bupivacaine/fentanyl labor epidurals.

435 ANONYMOUS URINE TOXICOLOGY SCREEN IN A RURAL STATE

RA Wright[X], F Byford[X], S Carter[X], MA Morgan, KM Parker[X], PC Toubas[X], D Blouse[X], Oklahoma Univ. Health Sciences Center, Oklahoma State Health Dept., Indian Health Services, Oklahoma City, OK and Univ. of California, Irvine, Orange, CA.

Although substance abuse in inner-city pregnant populations is reported to be high, the frequency of abuse in a rural state remains unknown. The purpose of this study was to anonymously screen urine specimens of consecutive deliveries during one month from three hospitals, in different cities and geographic areas of a rural state and determine perinatal outcomes. Specimens were collected on admission from 435 patients in labor (57% teaching hospital, 25% public hospital and 19% private hospital) and tested at a NIDA certified laboratory for amphetamine, barbiturate, benzodiazepine, marijuana, cocaine, opiate and phencyclidine. Positive screens were observed in 90 patients (86% one substance; 12% two; 2% three). Babriturate(51%) was most frequently seen, followed by opiate (19%), amphetamine (14%), marijuana (13%), benzodiazepine (2%) and cocaine (0.9%). However, the barbiturate positives were prescribed. The patients with positive and negative results were similar in age, parity, race, delivery gestational age, mode of delivery, neonatal birthweight, apgar scores, low birth weight and perinatal mortality. The three different participating hospitals had similar positive results. However, those positive for opiates were observed to deliver at a significantly earlier gestational age than negatives (37.9±3.6 vs. 39.2±2.3, p<0.025). Although these hospitals in a rural state have a similar frequency of positive toxicology screens at delivery as reported for inner-city hospitals, the distribution of substances is drastically different, with a low frequency of cocaine. Similar studies from other rural settings are needed to determine where to concentrate therapy efforts for substance abuse during perinatal period.

Poster Session V

Saturday, February 8, 1992
10:30 a.m.-12:30 p.m.

Grand Salons I-IV

CATEGORIES	POSTER NOS.
Clinical & Operative Obstetrics	436-493
Antepartum Fetal Testing	494-527
Neonatology	528-535

436 **ASYNCHRONOUS MULTIPLE BIRTH SYNDROME**

J.P. Lavery, M.D., R.J. Austin, M.D.
D.S. Schaefer, M.D.*, S. Aladjem, M.D.
Bronson Methodist Hospital
Kalamazoo, Michigan

Extending the interval between the births of members of a multiple gestation is uncommon. Prolongation of time in utero may allow for survival of the later delivering fetus(es) and also lower the long term morbidity of prematurity. We report four cases in which aggressive tocolysis, antibiotic therapy, liberal hospitalization and cerclage (3 cases) achieved a dramatic extension of the pregnancy after the primary delivery. Although all first delivered fetuses died, 4 of 5 of the remaining infants survived and are without major complications. Aggressive therapy has a place in selective cases of asynchronous births in multiple gestations.

CASE	GESTATION (WEEKS) 1ST.....2ND BIRTH		TIME GAINED	2ND DEL OUTCOME
1.	21	24	21 DAYS	NND
2.*	18 2/7	34 1/7	111 "	TWINS-A&W
3.**	26 3/7	29 4/7	20 "	A&W
4.	21 5/7	33 3/7	82 "	A&W

*Pregnancy began as a triplett gestation
**Stillbirth with first birth, no cerclage.
NND=Neonatal death; A&W=Alive and well without major complications.

437 PREDICTING SUCCESS IN A TRIAL OF LABOR. L.R. Troyer, V.M. Parisi. Dept. Ob/Gyn, University of Texas HSC, Houston, TX.

Increasing experience with vaginal birth after cesarean delivery (VBAC) attests to its overall success, but has also raised controversy as to its universal safety. The purpose of this study is to identify factors predicting success and/or failure in a trial of labor (TOL) and to determine if a subset of patients at high risk to fail a TOL consequently suffer greater morbidity. In a retrospective chart review from Jan. 1990 - Jan. 1991, 264 labors with documented transverse lower uterine segment scars were analyzed. Previous birth weight, diabetic status, and current estimated fetal weight did not affect the outcome of a TOL. Success of a TOL was significantly influenced by (1) previous dysfunctional labor, (2) no prior vaginal delivery (PVD), (3) nonreassuring admission fetal heart tracing (NR FHT), and (4) induction of labor (Table 1). A scoring system was then constructed to predict VBAC success rates, with one point assigned for each variable present at admission (Table 2).

TABLE 1

Variables	VBAC	C/S
Prev. dys. labor	63.4%*	36.6%
No PVD	67.4%*	32.6%
NR FHT	43.6%*	56.3%
Induction	52.9%*	47.1%

(*p < .05 compared to overall VBAC rate of 72.7%)

TABLE 2

Score	N	VBAC
0	59	91.5%
1	92	73.9%
2	87	66.7%
3-4	26	46.1%

The incidence of uterine rupture was 1.1% (n = 3), all in the C/S group. There was no increased maternal or fetal morbidity between VBAC and C/S in those patients scoring 3-4 points. We conclude: (1) based on the additive presence of each variable, we have identified subsets of patients undergoing a TOL who have high rates of success and high rates of failure; (2) a TOL in the subpopulation of patients with the lowest percentage for success (score 3-4) does not increase maternal morbidity; and (3) this scoring system may influence the counselling of patients who desire a TOL.

438 **VAGINAL BREECH DELIVERY IN THE 1980s-*PRETERM GESTATION.*** C.Weiner and L.Estle*, Univ Ia College of Med, Iowa City, Ia 52242

Vaginal delivery (VagD) of the selected breech remains clinically controversial. Criticisms have included, sample size, the % of patients allowed a trial, delivery prior to widespread use of epidural anesthesia, fetal monitors, and umbilical blood gases. To resolve many of these issues, we examined the mother/child hospital records of 850 breech and 1611 vertex singleton deliveries between 1981 to 1990. This abstract focuses on the 223 with completed records <36w. 19 (8.6%) had lethal and 41 (20%) nonlethal abnormalities; each higher than the matched vertex neonates (each $p<0.05$). Excluding neonates with lethal defects, 55% (112/204) had a trial of labor. 69% of these were either frank or complete. Selection criteria were staff dependent and included a flat plate, pelvimetry, a Zatucchni-Andros score and an ultrasound weight estimate. 71% delivered vaginally (40% overall, 90% by resident staff). The PNM rate appeared higher for all VagD (V:173/1000; CS: 82/1000, $p=.08$) and after exclusion of 7 neonates <25w (V:147/1000; CS:67/1000, $p=.11$). Neither GA at delivery (V:30±4w; CS:31±3w), the GA of those that died (V:26±2w; CS:27±3w), the incidence of significant trauma nor metabolic or respiratory acidoses varied significantly by route. *Because of the large sample, GA could be stratified. PNM did not differ by route from 32 to 36w (V: 0/1000; CS: 19/1000,p=.83). It was higher for VagD <32w (V:275/1000; CS:106/1000, p=0.05). RDS was less frequent after VagD (V:46%; CS:62%, p=0.05), a benefit not confined to any particular GA. The incidence of IVH was increased by VagD, but the increase was confined to those < 32w (V:31%; CS:11.8%, p=0.02).* **CONCLUSION: The breech ≥ 32w does not benefit from routine caesarean delivery and is at greater risk of RDS if delivered by caesarean. Therefore, routine caesarean delivery of the breech ≥ 32 w cannot be medically justified. In contrast, caesarean delivery of the breech < 32 w reduces the PNM predominantly by a reduction in IVH.**

439 **VAGINAL BREECH DELIVERY IN THE 1980s- *TERM GESTATION.*** C.Weiner and L.Estle*. Univ Ia College of Med, Iowa City, Ia 52242.

Vaginal delivery (VagD) of the selected term breech remains clinically controversial despite 2 randomized trials and several large retrospective studies confirming safety. Criticisms have included sample size, the % of patients allowed a trial, and delivery prior to widespread use of epidural anesthesia, fetal monitors and umbilical blood gases. To resolve many of these issues, we examined the mother/child hospital records of 850 breech and 1611 vertex singleton deliveries between 1981 to 1990. This abstract focuses on the 462 breeches ≥36w. 4 (.9%) had lethal and 110 (24%) nonlethal anomalies; each higher than the matched vertex deliveries ($p<0.05$). After excluding neonates with lethal defects, 69% (316/458) had a trial of labor. Selection criteria were staff dependent and included a flat plate, pelvimetry, a Zatucchni-Andros score and an ultrasound weight estimate. X-ray pelvimetry was done in only 35% and altered the plan in 19% of those. 63% delivered vaginally (43% overall, 84% by resident staff) (3153±401g, range 2060-4350). ***There was only 1 perinatal death -a patient who presented with the fetus on the perineum without a heart rate (corrected PNM 0/1000).*** Though a metabolic acidosis was more common after labor and VagD (V:6.3%; CS:1.1%, $p=.02$), there were no clinical sequellae. Neonatal trauma (includes bruising and laceration) was greater after VagD (45 vs 8%, $p<0.0001$) and a nuchal arm (6.1%) was seen only with VagD. *However, the incidence of significant trauma (fractures, nerve palsies, etc) did not vary by route (V:2.1%; CS:3.3%, p=NS).* Further, among VagD patients, an epidural was associated with a decrease in total (50 vs 38%) but not significant trauma. The incidence of trapped head did not vary by route (V:2.6; CS:.8, $p=.3$). VagD was unassociated with NICU admission or RDS (V:1.5%; CS:4.2%). However, maternal morbidity and hospital days were each increased by CS ($p<0.001$). **CONCLUSIONS: *Routine* caesarean delivery of the term breech is not medically justifiable.**

440 **ACTIVE MANAGEMENT OF LATENT LABOR WITH UNKNOWN UTERINE SCAR INCREASES RISK OF UTERINE RUPTURE.** D.K. Grubb,[X] S.L. Kjos, R.H. Paul. University of Southern California, Los Angeles, CA

Trial of labor is now frequently undertaken after prior cesarean birth. The term patient with an unknown uterine scar and persistent uterine contractions presents an unknown risk of uterine rupture. A prospective, randomized study was undertaken to determine if prolonged labor and operative delivery could be minimized by outpatient observation until labor was confirmed. Uncomplicated, term patients with one or two prior cesarean births were randomly assigned to active inpatient management, with oxytocin augmentation for persistent contractions, or to outpatient evaluation and observation until labor was ruled in. Of 197 patients enrolled, 8 failed to return for delivery, and were excluded from analysis. Four initially requesting trial of labor elected repeat cesarean section after enrolling.

	Expectant (94)	Active (95)	p
2 prior cesarean births	20 (21%)	16 (17%)	NS
Prior vaginal birth	31 (33%)	31 (33%)	NS
Cesarean delivery	17 (18%)	15 (16%)	NS
Oxytocin used	49 (52%)	76 (80%)	0.0001
Hours active labor (median)	4.25	4	NS
Uterine scar disruption	0 (0%)	5 (5%)	0.03

Four cases of asymptomatic low transverse uterine scar dehiscence and one case of vertical uterine scar rupture requiring hysterectomy occurred. All five were in the inpatient active management group, and all had received oxytocin augmentation. We conclude that, in term gravidas with unknown uterine scars exhibiting persistent uterine contractions, expectant management did not reduce the rate of cesarean delivery or prolonged labor. Second, expectant management was associated with a decreased usage of oxytocin. Finally, the risk of uterine scar dehiscence or rupture was increased significantly by active inpatient management compared to expectant outpatient management.

441 **CLINICAL CHARACTERISTICS AND NEONATAL OUTCOME IN PATIENTS WITH PRETERM PREMATURE RUPTURED MEMBRANES WHO DELIVER WITHIN 72 HOURS.** S.J.Carlan,J.V.Parker*,U of S FL,Tampa,FL,ORMC,Orlando,FL

Over a two-year period 386 patients with preterm premature ruptured membranes (rupture of membranes prior to labor < 37 weeks gestation) delivered at Tampa General Hospital. Of these, 19 terminated electively and 14 resealed. Of the remaining 335, 205 (61%) delivered within 72 hours. No patient was tocolyzed. The group that delivered within 72 hours was similar in GTPAL, racial makeup, incidence of smoking, and positive cervical cultures for Gc and GBBHS to the group that delivered after 72 hours. The incidence of multiple gestations, breech presentations, abruptios and fetal distress in labor was also not significantly different between the groups. Neonatal outcomes were similar in incidence of NEC, IVH, and positive blood cultures.

CLINICAL CHARACTERISTICS AND OUTCOME PARAMETERS (MEAN ±1SD)

	Delivery <72 hrs N=205	Delivery >72 hrs N=130	P
Age (yrs)	24.1 ± 6.6	23.5 ± 5.5	NS
EGA at rupture (wks)	32.5 ± 3.5	30.9 ± 3.7	<.05
Cx Clamydia + (%)	5.3	13.8	<.05
Initial US deepest pocket (cm)	2.6 ± 1.6 (N=156)	3.1 ± 1.6 (N=126)	<.05
Amnio + gr stain;#(%)	11/36 (30)	6/59 (10)	<.05
Clinical chorio (%)	14	18	NS
Pit induction (%)	8.2	12.3	NS
C-Section (%)	19.5	25	NS
Newborn wt. (grs)	1999 ± 661	1879 ± 714	NS
ROM to delivery (d)	1.07 ± 0.7	11.9 ± 14.1	<.05
Cord pH	7.29 ± 0.08	7.31 ± 0.09	NS
Newborn Respirator (d)	1.9 ± 6.3	3.9 ± 12.3	.05
NICU (d)	10.7 ± 19.3	17.4 ± 28.7	<.05

We conclude that women with earlier gestational ages, deeper amniotic fluid pockets, and a lower incidence of positive amniotic fluid bacterial studies are likely to delay delivery > 72 hrs. Inspite of a mean latent period of almost 12 days and similar gestational ages at delivery, the neonates in the delayed delivery group had more ventilator and NICU days.

442 **THE FISCAL IMPACT OF THE MEDICAID ABORTION FUNDING BAN IN MICHIGAN.** MI Evans, E Gleicher, MP Johnson, RJ Sokol, Dept Ob/Gyn, Wayne State University/Hutzel Hospital, Detroit, MI

Attacks upon the availability of abortion have occurred in many states. In Michigan after several attempts, a 'no Medicaid funding of abortion law' went into effect in December, 1988, prior to which the number of abortions per year in Michigan and the number of abortions per 1000 residents remained relatively steady.

Year	# Abortions Reported	# Abortions/ 1000 Livebirths	Deliveries
1987	49,098	340.4	140,466
1988	46,747	331.2	139,635
1989	36,557	248.4	148,164
1990	36,183	236.0	153,304

Following the change in the law the number of abortions decreased by about 10,000 which was closely reflected in increased births. Such an increase was not seen in surrounding states. At Hutzel Hospital, the largest delivery service in Michigan, deliveries rose 10% in 1989 and 7% in 1990. However, while the overall percentage of Medicaid patients remained steady at approximately 50% in our hospital (an increase of 600 Medicaid deliveries), the percentage of NICU Medicaid volume rose from 64 to 68%. These findings are consistent with the supposition that unwanted pregnancies result in neglected prenatal care. Poor care gives rise to prenatal complications and NICU admissions which may have been avoided had freedom of access to abortion services been an option for these women. It is unknown just how many of these pregnancies would have continued, but using the cohort of an additional 13,669 Michigan babies born in 1990 over 1988 numbers at an average medical cost of $3708 (Medicaid estimate) for the first year, the overall cost will be $54,748,620. There have been no increased appropriations by the Michigan legislature to care for these babies.

443 **EARLY REPAIR OF EPISIOTOMY DEHISCENCE ASSOCIATED WITH INFECTION.** R. Ramus[X], S. Ramin, B. Little[X], L. Gilstrap, Dept. Ob/Gyn, Univ. Texas Southwestern Med. Ctr., Dallas, TX

The traditional approach to episiotomy dehiscence has been delayed repair. Recently, early repair of episiotomy dehiscence has been examined in a military population. The purpose of the present study was to examine early repair in a city-county hospital setting serving predominantly an indigent population. The incidence of episiotomy breakdown at our institution was 0.5%. Our policy, since September 1, 1989, has been to proceed with early repair in the immediate postpartum period. Medical records were reviewed on 34 of 35 patients who have undergone early repair. Of these, 21 (62%) had midline and 13 (38%) had mediolateral episiotomies. Twenty of the former and 8 of the latter group had a third-degree or fourth-degree extension. Dehiscence was associated with episiotomy infection in 27 (79%) of the 34 patients -- 18 (86%) in the midline and 9 (69%) in the mediolateral group. All patients received antibiotic therapy and wound care prior to repair. In addition, those with fourth-degree episiotomy breakdowns received a go-lytely bowel prep. Repair was accomplished from 3 to 13 days ($\bar{x}$=6.4) following dehiscence. Successful repairs were accomplished in 32 of 34 (94%) patients. Two patients (6%), with an initial third-degree episiotomy, had a subsequent breakdown of their repair and were allowed to heal by secondary intention. In conclusion, most dehiscences in our population are associated with infection. Unlike previous reports, infection was as common in midline as in mediolateral episiotomy dehiscences. Early repair of episiotomy dehiscence in this population is associated with a satisfactory outcome in the vast majority of patients.

444 **TRANSVERSE UTERINE INCISION CLOSURE: ONE VERSUS TWO LAYERS**

J.C. Hauth, J. Owen, R.O. Davis, T. Lincoln[x], J. Piazza[x]
University of Alabama Hospitals, Birmingham

In 1926, Munro Kerr described the transverse lower uterine segment incision and recommended a two layer closure. Theoretically, a one layer closure should disrupt less tissue, introduce less foreign material, require less operative time, and perhaps achieve hemostasis more rapidly. To test this hypothesis, we prospectively randomized 761 women to closure with either one continuous layer of a locking #1 chromic gut suture using a CTX needle (#384) or two continuous layers of #1 chromic gut with the first layer locked (#377). Both groups had similar demographic and intrapartum risk factors. Women who had a one layer closure required less operative time, 39 versus 45 minutes, (p=.004) and fewer uterine hemostatic sutures, a mean of 0.65 versus 0.82 for one and two layers respectively (p=0.03). Endometritis (excluding patients with chorioamnionitis) was similar in both groups (21 vs. 16% p=0.34) and a Hct decrease of ≥ 8% from the pre- to postoperative day one occurred in 11.7% (one) vs. 15.5% (two) p=.14. In no outcome assessment was the two layer closure of more benefit than the one layer. We recommend that a transverse incision be placed in the true lower uterine segment and that a one layer closure be used when anatomically feasible.

445 **SOCIOBEHAVIORAL CHARACTERISTICS AND INTRAUTERINE GROWTH RETARDATION: A MULTIVARIATE ANALYSIS.** L.C. Castro, C. Hobel, L. Platt. Dept OB-GYN, Cedars-Sinai Med Ctr. LA, CA.

The purpose of this study was to determine the prevalence rates for tobacco use (TU) & substance abuse (SA) in a broad spectrum of pregnant women & to evaluate the individual & interactive effects of TU, SA & sociodemographic characteristics on intrauterine growth retardation (IUGR). **Methods:** 8,914 women delivering in a university affiliated hospital from 1986-90 were studied. Information on TU, SA (use of alcohol, marijuana and other illicit drugs) & sociodemographics was obtained antenatally. IUGR was a birthwt < 10th% for gestational age (GA). Univariate logistic regression was used to assess the association between each sociobehavioral factor & IUGR. Stepwise multivariate analysis was used to determine their interactive effects on IUGR. **Results:** Prevalence Rates

Race-ethnicity	White	Black	Asian	Hispanic	p-value
Tobacco use	17%	13%	8%	5%	<.0001
Substance abuse	7%	7%	5%	3%	<.0001
Insurance status	Uninsured	HMO	Private	p-value	
Tobacco use	14%	8%	5%	<.001	
Substance abuse	6%	5%	4%	<.01	
Marital status	Single	Married	p-value		
Tobacco use	17%	8%	<.0001		
Substance abuse	9%	4%	<.0001		

TU & SA were lowest in 30-39 year old women (6% & 4%) vs other ages (p<.001). Univariate analysis showed that TU, alcohol, marijuana, race & non-private insurance were significantly associated with IUGR. Multivariate analysis showed that TU (odds ratio= 1.96, p<.0001), marijuana use (odds ratio=1.69, p<.03) & race (odds ratio= 1.66 for blacks, p<.001) were most strongly associated with IUGR. For the categories SA, TU, & SA plus TU the prevalence of IUGR progressively increased (p<.0001) & this increase was greatest in black women. **Conclusions:** There are significant interactive effects of TU, SA & race on IUGR. Supp. by UCTRDRP.

446 **PREDICTORS OF SUCCESS IN THE EMERGENT CERCLAGE.** T.F.Kelly MD,[x] L.R.Troyer MD, K.M.Piacquadio MD,[x] C.J.Cantrell MD, V.M.Parisi MD,MPH, T.R.Moore MD. *From the Divisions of Maternal Fetal Medicine of the University of California, San Diego and the University of Texas at Houston and the Department of Obstetrics and Gynecology, Balboa Naval Hospital, San Diego.*

It is difficult to counsel the mid trimester patient who presents with advanced cervical dilatation. Available literature does not delineate the variables most prognostic for the success of emergent cerclage. We retrospectively reviewed charts of 20 patients (21 fetuses) who presented within the last 6 years with advanced cervical dilatation and visible or prolapsing membranes, in the second trimester. The average gestational age (GA) on admission was 20.9 ± 3 (SD) weeks (wks), weeks gained were 8.9 ± 7.4 wks and GA at delivery was 30 ± 7.7 wks. Neonatal survival correlated negatively with cervical dilatation on admission (R=0.46, P<0.04). If cervical dilatation was > 3.5 cm, survival was 0% (n=3); if ≤ 3.5 cm survival was 83% (n=18) (P=0.015). Cervical effacement and membrane prolapse on admission were not predictive of weeks gained. Of 8 who had prolapsing membranes, 80% went greater than 50 days from cerclage to delivery. There was no significant relationship between time from admission to surgery or the time on tocolytics to weeks gained. We conclude that (1) cervical dilatation > 3.5 cm is prognostic of poor outcome in the performance of emergent cerclage, (2) neither cervical effacement nor evidence of membrane prolapse were predictive of successful outcome, and (3) use of tocolytics prior to cerclage appears not to improve outcome.

447 **THE MANAGEMENT OF BREECH PRESENTATION: CESAREAN SECTION VERSUS VAGINAL DELIVERY**

Tracy L. Wells[x], Luis Sanchez-Ramos, M.D., M.D., Mark T. Cullen, M.D. Division of Maternal-Fetal Medicine, University of Florida, Jacksonville, FL

The management and perinatal outcome of 451 cases of singleton breech presentation occurring at 34 or more weeks' gestation and with a fetal weight greater than 2000 grams are reviewed. Beginning in 1986 a trial of labor was offered if the breech was frank or complete, had an estimated weight between 2000 and 4000 grams, adequate pelvimetry, and a non-extended fetal head. Three hundred-thirty patients underwent a cesarean section without a trial of labor; most of these cesarean deliveries were for patients' "choice". The incidence of elective cesareans because of this indication decreased from 62% to 6% in a five year period. Of the 121 patients who qualified for a trial of labor, 22 had a cesarean section, and 99 had a successful vaginal delivery. Approximately 80% of patients who met protocol criteria delivered vaginally. Comparing the 330 patients with an elective cesarean delivery to the 99 who delivered vaginally revealed no difference in several outcome parameters including: NICU admissions, Apgar scores less than 7 at 1 and 5 minutes, umbilical cord gases, and birth trauma. There was a significant increase in maternal morbidity and length of hospital stay in the cesarean section group. We conclude that a trial of labor in selected patients can be achieved without an increase in perinatal or maternal morbidity and mortality.

448 CHOICE OF DELIVERY METHOD AFTER CESAREAN SECTION. A Stek* MD, R Jacobson MD, J Khoury*, Department of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, Ohio

Despite recent recommendations, vaginal birth after cesarean section (VBAC) is still not completely accepted as a desirable option by many patients and physicians. Our study was therefore designed to determine the factors which may influence patients' decisions regarding method of delivery after a previous cesarean section (C/S). 55 patients with a previous low transverse C/S were interviewed with respect to choice for method of delivery (trial of labor [TOL] versus repeat C/S) for the current pregnancy and the reasons for this choice. Spontaneously mentioned reasons were noted, after which the patients indicated the factors in their decision from a standardized list. 42 patients (76%) preferred TOL whereas 13 (24%) desired a repeat cesarean section. These two groups were similar in age, race, gravidity and parity. Patient reasons for preferring TOL were: (a) comfort and convenience (52%), (b) desire to experience a normal birth (43%); and (c) physician/midwife advice (24%). Using Fisher's Exact Test and Chi-Square analysis, specific factors which were statistically significant for TOL choice included: decreased postpartum pain, shorter recovery and hospitalization, current and previous physician's advice, desire to experience a normal birth and avoid surgery, remain in control of the birth process, decreased anesthetic and medication use and decreased risk of infection or surgical trauma, as well as history of a previous vaginal delivery, and problems experienced with a previous C/S. Patient reasons for preferring repeat C/S were: (a) comfort and convenience (100%), (b) safety for mother and infant (75%), and (c) experience with C/S and lack of familiarity with vaginal birth (42%). Educational level, insurance status and the partner's opinion were not statistically significant factors. In summary, the strictly medical and financial benefits of VBAC were not of major significance in the patients' decision making process. The current and previous physicians' advice however, did significantly influence the patients choice of method of delivery. We propose that physician counseling at the time of the original C/S and subsequently can contribute towards further lowering of the rate of repeat cesarean sections.

449 **MID-TRIMESTER PREGNANCY TERMINATION: A RANDOMIZED TRIAL OF PROSTAGLANDIN E2 VERSUS CONCENTRATED OXYTOCIN**

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Concentrated oxytocin infusion was compared with prostaglandin E2 (PGE2) vaginal suppositories for indicated mid-trimester (17-24 weeks) abortion in a prospective, randomized analysis. **Methods**: 72 patients received either PGE2 (N=36) or concentrated oxytocin (N=36). The uterine size was ≤ 24 cm in all patients. Treatment consisted of six PGE2 suppositories (one q4h) or six cycles of an escalating concentration of oxytocin (50X units in 500 cc of normal saline q4h - X represents the cycle number). Unless delivery was imminent at 24 hrs, the agent was considered to have failed, and patients were crossed to the other method. **Results**: Indications were similar between the two methods (p=0.38) and included fetal anomalies (N=36), fetal death (N=17), PROM (N=14), and maternal indications (N=5). The patients were of a similar age, parity, race, gestational age, uterine size, and initial cervical dilatation. There were 12 first-round failures: 5 (14%) with PGE2 and 7 (19%) with oxytocin, p=0.53. Of these, 11 were delivered during the second 24 hrs, and one patient required treatment beyond 48 hrs. Failures were significantly related to gestational age (mean 18.5 weeks in failures versus 19.8 weeks in the successes, p=.001) and anencephaly (3 of 6 vs. 4 of 66, p=.01). Considering the failures and subsequent crossovers, a total of 85 patient-trials were completed. Maternal side effects were more frequent with PGE2: fever (p<.001), nausea (p=.004), vomiting (p<.001), and diarrhea (p<.001). No patients experienced neurologic symptoms related to hyponatremia. Pre- and post-treatment electrolyte values were similar in 14 patients. **Conclusions**: Concentrated oxytocin is a satisfactory alternative to PGE2 for indicated mid-second trimester abortions.

450 **VACUUM EXTRACTION IN PRETERM INFANTS: A CASE CONTROL STUDY**

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The vacuum extractor is now an established instrument in the practice of obstetrics. However, there is paucity of data on the application of vacuum extraction in preterm deliveries. This study was designed to determine perinatal and developmental outcomes in preterm infants (34.2 ± 1.9 weeks; 2080 ± 387 gm). delivered by vacuum extraction compared to spontaneously delivered controls (33.6 ± 2.3 weeks; 2071 ± 357 gm) From January 1989 to June 1991, 47 infants were delivered by vacuum extraction. Ninety-eight well matched controls were selected for comparison. During the study period there were approximately 13,104 deliveries at this institution. Both groups were matched for: maternal and neonatal demographics, indications for delivery, and labor characteristics. No statistically signficant difference was noted in Apgar scores, umbilical cord gases, length of NICU stay, length of hospital stay, number of NICU admissions, and incidence of retinal and intraventricular hemorrhages. Developmental outcome was evaluated and did not appear to be affected by instrumental deliveries. Our data support the indicated use of vacuum assisted delivery in preterm infants.

451 **COUNSELING BASED ON OBSTETRICAL HISTORY: RECURRENCE RATES FOR ABNORMALITIES OF BIRTH WEIGHT, GROWTH, AND GESTATIONAL AGE.**

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There is often little information to guide counseling based on obstetrical history. Many of the available studies calculate recurrence retrospectively using multiple/single occurence ratios, which imprecisely estimate both followup rates and the number of cases at risk. This report addresses the lack of recurrence studies for macrosomic, postterm, and large for gestational age births. We analyzed 4349 women with more than one singleton live birth at our hospital from 1984-1990 and calculated the frequencies, odds ratios, and 95% confidence intervals (CI) tabulated below.

	Incidence	Recurrence	Odds(CI)
>4000 gm	5.1%	30.6%	8.2(6.0-11.3)
<2500 gm	15.7%	47.7%	4.9(4.1-5.8)
≥42 wks	6.6%	12.3%	2.0(1.4-2.9)
<37 wks	13.1%	42.9%	5.0(4.1-6.0)
LGA	8.4%	33.8%	1.5(1.1-2.1)
SGA	8.9%	20.3%	1.5(1.1-2.0)

We believe the high recurrence rate for prematurity and low birth weight may be in part a reflection of our referral pattern. Recurrence rates for macrosomia and posterm gestation are subject to variation in obstetrical management; these rates would be higher without intervention. Clinicians should consider individual and institutional practice patterns as well as the above rates when counseling patients.

452 ANTEPARTUM AUTOLOGOUS BLOOD DONATION: COST/BENEFIT ANALYSIS. CA Combs MD PhD*, EL Murphy MD MPH*, RK Laros, Jr., MD. University of California, San Francisco

To minimize the risk of infection after homologous blood transfusion (TX), autologous blood donation (AuBD) has been recommended before procedures with a high risk of TX. Antepartum AuBD has been shown to be safe for mother and fetus in patients with "traditional" risk factors for obstetric TX. However, the majority of units so donated are not needed for TX, except in patients with placenta previa. We attempted to develop rational recommendations for antepartum AuBD based on actual risk factors for TX. We reviewed the TX experience in 14,267 consecutive term deliveries from 1978-88. Women with placenta previa were excluded. Preterm deliveries were excluded because these women could not have completed an AuBD program. Ten risk factors that could have been known in advance were studied. On multiple logistic regression, four factors were significantly (P<0.05) associated with TX: preeclampsia (odds ratio=3.7), multiple gestation (OR=2.8), elective cesarean (OR=1.7), and nulliparity (OR=1.5). There was no association between TX and ethnic group, prior abortions, prior cesarean section, or prior postpartum hemorrhage. In a cost/benefit analysis, we assumed that the cost of a 2-unit AuBD was $100, the risk of hepatitis 1 in 20 homologous units, and the risk of HIV infection 1 in 40,000 homologous units. Lower infection rates would result in higher costs per case prevented by AuBD.

# of Risk Factors	# of Pts. Transfused	Cost of AuBD to Prevent One Case of: Homologous TX	Hepatitis	HIV
0	43/6218 (0.7%)	$23.0K	$161.8K	$300.0M
1	83/7187 (1.1%)	11.6K	88.0K	136.9M
2	24/810 (3.0%)	4.3K	35.2K	73.6M
3 or 4	4/52 (8.0%)	1.3K	13.3K	26.0M

CONCLUSION: In obstetric patients without placenta previa, the probability of TX or TX-related infection is too low for antepartum AuBD to be cost-effective.

453 **OUTPATIENT PROSTAGLANDIN E2 SUPPOSITORIES IN POSTDATES PREGNANCIES.** SK Sawal, WF O'Brien, MS Mastrogiannis, MG Mastry*, GW Porter*, L Johnson*. University of South Florida, Tampa, Florida

Although the safety of low dose outpatient PGE2 for postdate pregnancy has been established (Obstet Gynecol 78:19, 1991), the efficacy and advantages remain unknown. We investigated daily self-administered 2 mg PGE2 vaginal suppositories in accelerating cervical ripening in this double blind, placebo controlled study. 72 patients with uncomplicated pregnancies ≥ 41 weeks gestation and a Bishop score of < 9 received either 2 mg PGE2 placebo or vaginal suppositories. The groups were comparable in age, parity, EGA, and Bishop score. Patients were admitted for labor induction if the Bishop score was ≥ 9, for unfavorable antepartum test results, or other obstetrical complications. There were no cases of stimulation of regular uterine contractions or reports of other side effects. The results were

as follows	CONTROL(40)	PGE2(32)	p
Bishop score on L&D admission	11	10	NS
EGA on L&D admission (days)	298	295	<.05
No. of suppositories	8	2	<.05
Total oxytocin used (mU)	2192	484	<.05
Time in L & D (hrs)	12.2	8.4	<.05
C-sections	5	2	NS
Antepartum testing costs ($)	664	506	<.01

CONCLUSIONS: 1. Daily 2 mg PGE2 suppositories accelerate cervical ripening resulting in earlier spontaneous or scheduled admission for labor 2. Low dose PGE2 may decrease intrapartum cervical resistance as reflected by shortened labor course & decreased requirement for oxytocin, not necessarily reflected in the Bishop score.

454 **CESAREAN BIRTH SOLELY TO PREVENT MECONIUM ASPIRATION SYNDROME UNWARRANTED.**
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The persistence of meconium aspiration syndrome (MAS) with meconium staining despite intrapartum suction has led some clinicians to perform cesarean birth to prevent gasping in cases with little evidence of fetal distress. To investigate the relationship between mode of delivery and MAS, we studied 2,523 consecutive singleton, vertex, live births complicated by meconium stained fluid delivered in 1987-89. Of these, 172 had a final clinical diagnosis of MAS. MAS was associated with Apgar score of 0-3 at 1 minute ($p<0.001$), fetal scalp pH < 7.20, ($p<0.01$) and primary, repeat, and elective repeat cesarean delivery ($p<0.01$ in each case). Discriminant function analysis revealed no relationship to mode of delivery in the absence of a low Apgar score or scalp pH. Dysfunctional labor, abnormal fetal heart rate patterns, and the duration of labor were not significantly related to MAS. There was a 6.8% incidence of MAS with meconium staining that increased to 28.4% with an Apgar score of 0-3 or scalp pH < 7.20. This is in sharp contrast to the reported 4% frequency of aspiration among meconium stained stillbirths, and suggests MAS is linked mainly to acidosis at the time of birth rather than before birth. We conclude that cesarean birth to prevent intrauterine gasping is unwarranted; severe acidosis should be avoided irrespective of the presence of meconium.

455 **A PROSPECTIVE STUDY OF THE 30 MINUTE RULE IN THE TIMING OF CESAREAN DELIVERY FOR FETAL DISTRESS.** T.R. Moore M.D., W.M. Gilbert M.D., R. Resnik M.D., R.C. Stevenson M.D.* *Division of Perinatal Medicine, University of California San Diego, CA.*

A limit of 30 minutes from the recognition of fetal distress until delivery is a commonly recognized standard, yet supporting data are lacking. We prospectively studied the effect of timing of cesarean section (decision-to-delivery time (DDT) and OR-to-delivery time) on neonatal outcome in 261 consecutive cesarean sections performed for fetal distress (FD) from 12/85 to 2/88. The time of onset of labor, the time of recognition of fetal distress, the FHR abnormality/scalp pH, time to OR , time of delivery, umbilical gases (UBG), and Apgars were recorded on a data sheet validated by independent review the following day. The mean DDT was 31 ± 27 (SD) minutes. FD deliveries were divided into DDT<30' (66%) and >30' (34.%). The mean arterial UBG pH (7.23 ± .09 vs 7.25 ± .08, $p<.02$), venous pH (7.28 ± .08 vs 7.31 ± .07, $p<.004$) were statistically but not clinically different. Fetal acidemia (arterial pH<7.15, venous pH<7.20), was more frequent in the DDT<30' group (16% vs 7%, $p<.001$), but the incidence of Apgar 5'<7 (3% vs 1%) and admission to NICU (20% vs 21%) was similar. This study suggests that, utilizing traditionally accepted indicators of fetal distress, immediate neonatal outcome is not influenced by the decision to delivery time.

456 SUBSTANCE ABUSE IN PREGNANCY, A RURAL PERSPECTIVE. S.C. Fee, P. Meier, Dept. Ob/Gyn, Marshfield Clinic, Marshfield, WI.

Substance abuse in pregnancy is a well documented problem and has been associated with numerous perinatal problems. Most studies have noted a prevalence of 11-15%, but these studies have all relied on urban populations. We have attempted to address the problem of substance abuse in rural pregnant women by conducting urine toxicology screens on 714 pregnant patients in a rural obstetrical clinic. In the first 4 months of this ongoing clinical study we obtained anonymous urine toxicology screens from 301 consecutive women seen for their initial prenatal visit and from 413 consecutive women admitted to the labor/delivery suite. All urines were screened for cocaine, cannabinoids, opiates, barbiturates, benzodiazepams and amphetamines. All positive screens underwent confirmatory testing. The presence of secobarbitol in labor/delivery patients (n=4) was considered iatrogenic and was not included in the calculations. The prevalence of positive urine screens was 1.7%. Of patients presenting for prenatal care, 1.3% were positive. Labor/delivery patients had a prevalence of 1.4%. The substances found were as follows: opiates 1.3%, barbiturates 0.1%, cocaine 0.3%, cannabinoids 0.3%, 3 screens were positive for more than one substance. No screens were positive for amphetamines or benzodiazepams. Our findings suggest that substance abuse in pregnancy may not be as wide spread as suggested by previous studies and that the concept of universal screening for substance abuse in pregnancy may not be a cost effective measure in all populations.

457 POSTPARTUM MORBIDITY AFTER FOURTH DEGREE PERINEAL REPAIR. Kenneth G. Goldaber,[X] Paul J. Wendel,[X] George D. Wendel, Jr. Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX

Fourth degree extension of episiotomies and perineal lacerations can have serious sequelae. However, there is little data regarding the incidence of fourth degree repair morbidity: infection and dehiscence. We sought to investigate the frequency of puerperal complications in women with fourth degree perineal lacerations and episiotomy extensions. The hospital records of 389 women (2% of vaginal deliveries) in 1989 and 1990 who had fourth degree perineal repair were reviewed. The delivery room surgical technique involved layered closure with 00 and 000 chromic catgut suture. Twenty women (5.1%) had infection and/or dehiscence. Thirteen women (3.3%) had infected perineal repairs, and 10 of the repairs (77%) subsequently became dehiscences. Seven women (1.8%) had perineal dehiscence without infection. Overall, 17 dehiscences (4.4%) occurred, accounting for 85% of the postpartum morbidity. Seventeen of the women (85%) were primiparas, and 19 (95%) had episiotomy extensions. The mean birth weight in the group with morbidity was 3500 grams. When compared to the 369 women without repair complications, there were no significant differences between maternal age, race, parity, weight, smoking or human papilloma virus infection. Similarly there was no difference regarding duration of second stage of labor, occiput positions, forceps delivery, blood loss, macrosomia, or birthweight. The only significant association was with shoulder dystocia which occurred in 19 of 369 women (5.1%) without morbidity and 4 of 20 women (20%) with morbidity (P= 0.02). Conclusion: Postpartum morbidity after fourth degree perineal repair is an uncommon event, usually accompanied by perineal dehiscence. Unfortunately, fourth degree complications are not predicted by readily preventable antepartum or intrapartum factors.

458 **PERIPARTUM HYSTERECTOMY: A RETROSPECTIVE REVIEW**
Lorraine Stanco[X], M.D., David Schrimmer, M.D. Richard Paul, M.D.
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From January 1, 1985 to July 1, 1990 at LAC+USC Women's Hospital, there were 85,841 births (71,845 vaginal and 13,996 cesarean). Retrospective review of medical records and departmental statistics revealed 125 cases of either cesarean or immediate post partum hysterectomy, with 60 being total and 65 subtotal. The incidence of peripartum hysterectomy was 1.5/1000 births, with an incidence following vaginal delivery of .097/1000 whereas the incidence associated with cesarean birth was 8.4/1000 or one hundred times greater. Median parity was 2 and median gestational age was 38 weeks. In 81 cases (65%), there was a history of prior cesarean delivery. Delivery was by cesarean section in 118 pts., while 7 delivered vaginally. Placenta previa was the indication for cesarean in 58/118 (49%) pts. Of the 7 pts. delivered vaginally, the 4 with prior cesarean delivery had uterine rupture requiring hysterectomy, despite the fact that 3 of them had undergone prior successful VBAC. Indication for hysterectomy was the diagnosis of placenta accreta in 55 pts, uterine atony in 25 pts, unspecified uterine bleeding in 19 pts, uterine rupture in 14 pts, placenta percreta in 6 pts, fibroids in 5 pts and infection in 1 pt. The pathological diagnosis of accreta or percreta was confirmed in 26/61 cases (43%). In 108/125 cases (86.4%) estimated blood loss was at least 2000 cc (range 900-21000) and blood transfusion occurred in 102/125 pts (82%). Fifty-seven pts. were transfused more than 2 units and of these 27 received more than 10 units. There were no significant differences in blood loss or replacement products with respect to type or indication for hysterectomy. Maternal complications included 11 cases of infectious morbidity, 11 wound complications, 7 cases of coagulopathy, and 4 cases of profound hemorrhage with one resultant maternal death. Median birthweight was 3120 grams and 13/126 (10%) infants had a 5 minute Apgar score of less than 7. There were 2 intrapartum and 2 neonatal deaths, the latter being attributed to prematurity. Median discharge for mothers and infants was the fourth hospital day. Previous cesarean section, placenta previa and blood loss of 2000cc or more were identified risk factors leading to hysterectomy. For 37/125 (29.6%) pts., all three factors were identified.

459 INTRAPARTUM MANAGEMENT OF THE NONVERTEX SECOND TWIN. S.J. Sherman, B.W. Kovacs, Dept. Ob/Gyn, University of Southern California School of Medicine, Los Angeles, CA

The intrapartum management of twin gestations in the vertex-nonvertex presentation is controversial. The purpose of this study was to compare the following delivery methods of the nonvertex second twin with regard to neonatal outcome: assisted breech, breech extraction, external version, and cesarean section. From 1/89 to 1/90, 236 sets of twins were delivered: 109 vertex-vertex, 67 vertex-nonvertex, 59 nonvertex, and 1 unspecified. Among the 67 vertex-nonvertex, the findings were:

Second twin	Assisted breech	Breech extraction	External version	Cesarean section
N (%)	13 (20%)	26 (39%)	3 (4%)	25 (37%)
Birth weight	2314g	2387g	3140g	2399g
Apgar 5 min	8.3	8.3	8.2	8.6
NICU admit	0%	17%	0%	18%

In conclusion, there is no statistically significant increased neonatal morbidity for these specified methods of vaginal delivery of the nonvertex second twin compared to delivery by cesarean section.

460 THE EFFECT OF PLACENTAL MANAGEMENT AT CESAREAN DELIVERY ON BLOOD LOSS. C. M. McCurdy,Jr., [X]E. Magann,[X] C.J.McCurdy,[X] A. Saltzman,[X]Dept. Ob/Gyn, Naval Hospital Camp LeJeune, Jacksonville, NC.

The effect of alternative methods of placental delivery at cesarean section on blood loss has not been studied. We randomized and prospectively compared 62 gravidas with respect to manual or spontaneous placental delivery at cesarean section. Duration of the third stage of labor, duration of surgery, and antibiotic prophylaxis did not differ between the study groups ($p > .05$). Blood loss measured at cesarean delivery was greater in the manually delivered group, 967 ml ± 248 ml(n=31), compared with the spontaneously delivered group, 666 ml ± 271 ml, n=31, ($p < .0001$). The incidence of postpartum endometritis was 7-fold greater in the manual vs. the spontaneous group, 23 % vs. 3 % respectively ($p < .05$). We conclude that spontaneous delivery of the placenta through the uterine incision at cesarean delivery can result in less operative blood loss and a lower incidence of postoperative endometritis.

461 **CORRELATION OF CHORIOAMNIONITIS AND PLACENTAL ABRUPTION IN THE TERM GESTATION** Fadi Bsat, MD[X], Dept. Ob/Gyn, Eastern Virginia Medical School, Norfolk, VA

An association has been established between placental abruption in the preterm gestation and histologic chorioamnionitis. To determine if a similar association is present at term, forty-two pregnancies at ≥37 weeks gestation with clinically diagnosed placental abruption were compared to a control group of forty-two term pregnancies with no evidence of abruption. Pathological examination of the placentae was done on all the cases, specifically looking for findings of chorioamnionitis. Each case was then classified in one of three groups, depending on the severity of abruption, as determined by gross examination of the placenta (Table I). Mild and severe abruption were defined as <50% or >50% placental separation, respectively. Cases with no abruption (group 1) or mild abruption (group 2) had a similar incidence of histologic chorioamnionitis ($p=0.66$). Patients with severe placental abruption (group 3) had a higher incidence of chorioamnionitis than either group 1 or group 2 ($p<0.01$). Chorioamnionitis and placental abruption are correlated in the term gestation, but the chronological nature of this relation remains unclear.

TABLE I: Chorioamnionitis and abruption severity.

Group	Abruption severity	# patients	# Chorio-amnionitis	% Chorio-amnionitis
1	None	42	4	10
2	Mild	32	5	16
3	Severe	10	7	70*

* $p<0.01$ when compared to either group 1 or group 2.

462 CT PELVIMETRY IN MANAGING BREECH LABOR. M. Gimovsky, J. P. O'Grady, B. Morris[X], R. Petrie, Dept. of Ob/Gyn, Baystate Medical Center/Tufts University School of Medicine, Springfield, MA

Previous experience suggests that CT pelvimetry offers distinct advantages over conventional radiographic studies including less fetal/maternal irradiation, ease of interpretation, and greater accuracy. In a three year experience with 55 breech labors in which CT pelvimetry was utilized, 37/55 (67%) fetuses were delivered per vagina in an uneventful manner when adequate maternal pelvic measurements were noted. 15/55 (27%) had "borderline" measurements and were delivered by C/S for that indication. 2/55 (4%) labors had failure of descent followed by C/S delivery. One fetus (2%) evidenced hyperextension of the fetal head and was delivered by C/S. We conclude: CT pelvimetry was easy for the radiologist to interpret, added no additional expense to the patient, and provided the clinician with an improved radiographic estimation of pelvic dimension and architecture.

463 A COMPARISON OF THE STANDARD PAPANICOLAOU TEST AND THE *CERVEX BRUSH* IN PREGNANCY. P.A. Cook,[X] J. Wormsbaker,[X] J.E. Hamous,[X] V.D. Castracane,[X] Dept. Ob/Gyn, Texas Tech University Health Sciences Center, Amarillo, TX.

The Papanicolaou (PAP) smear is a widely used technique for cervical cancer screening. The presence of endocervical cells is a criteria for considering the cervical cytology sample adequate. This study was designed to compare the *Cervex-Brush* (Unimar) with the standard PAP smear during pregnancy. Cervical smears were obtained from each of 144 pregnant women (8-37 weeks gestation). Q-tip and Ayre spatula samples were obtained in our usual fashion and placed on a single slide. *Cervex-Brush* samples were obtained by placing the middle lines of the brush into the cervical os and rotating the brush in a clockwise fashion five times and placed on a separate slide. All slides were then evaluated by the same pathologist. Samples were obtained in a alternating fashion so that roughly one-half of the patients had the *Cervex-Brush* sample collected first and the other half had a standard PAP smear obtained first. Contemporary statistics from a separate group of 229 obstetrical patients, 50 (21.8%) had no endocervical cells. Of 144 initial smears, 88 (61.1%) of the smears obtained with the *Cervex-Brush* retrieved more endocervical cells than did the routine smear in the same patient. Only 18 (12.5%) of the routine smears retrieved more endocervical cells than did the *Cervex-Brush*. Twenty-nine smears (20.1%) had equal amounts of endocervical cells and in 9 (6.3%) there were no endocervical cells retrieved with either method. There seemed to be no increase in cervical bleeding associated with this procedure. Patients continue to be enrolled to reach a total of 200 subjects. This data indicates an improved method of endocervical cell retrieval during pregnancy with the *Cervex-Brush* and may become the method of choice for obtaining PAP smears in pregnancy.

464 PERINATAL SEPSIS AND DEATH ASSOCIATED WITH RETAINED CERCLAGE IN PATIENTS WITH PREMATURE RUPTURE OF MEMBRANES

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The timing for cerclage removal in patients with preterm premature rupture of the membranes (PPROM) is controversial. Early removal has been advocated due to concerns for perinatal infection. Recently, Yeast and Garite (AJOG 1988) found that early cerclage removal was not associated with an increased risk for immediate delivery or infection, however, the possible sequelae of retained cerclage were not addressed. We have, therefore, assessed the effect of removing or retaining cerclage in cases of PPROM. Prophylactic cerclages were performed in 246 patients at our institution over a 12-year period for a history consistent with cervical incompetence. Patients having cerclage placed for cervical change detected during the index pregnancy were excluded. Thirty-one singleton pregnancies associated with PPROM without labor between 24 and 32 weeks of gestation were identified. Four women were delivered upon presentation for overt chorioamnionitis. The remaining 27 patients were entered into an expectant protocol. Of the 27 patients, 21 (78%) had immediate cerclage removal (Group A); six women (22%) opted to retain the cerclage (Group B). There were no statistically significant differences between the two groups regarding gestational age at time of cerclage (15.3±1.9 weeks vs. 15.1±2.4 weeks, p=0.8); gestational age at PPROM (28.3±2.2 weeks vs. 27.7±2.8 weeks, p=0.5); birthweight at delivery (1320.76±495.25 gm vs. 1440.33±872.69 gm., p=0.6); the latency period (5 days vs. 3 days, 50th percentile), and maternal febrile morbidity (6/21 vs. 4/6, p=0.27). Immediate neonatal death occurred in 2 babies in group A (9%) compared to 5 in group B (83%) (p=0.006). All deaths were secondary to sepsis. We conclude that retaining cervical cerclage in cases of PPROM does not increase the latency period to delivery or birthweight but is associated with a dramatic increase in neonatal morbidity and mortality.

465 MATERNAL TRANSPORT OF PATIENTS WITH ADVANCED CERVICAL DILATATION -- TO FLY OR NOT TO FLY

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Emergency maternal transport of patients in advanced preterm labor often involves difficult decisions about whether to transport or not. Numerous studies have documented an increased survival rate, decreased short and long term morbidity, and decreased cost of hospitalization for infants transported in utero and delivered at a tertiary care facility. A retrospective review of maternal transports performed in Northern Arizona by Samaritan Air Evac Services was performed covering a 21 month period. Fifty-four (5%) of 1,080 patients transported for preterm labor were in advanced stages of labor (≥ 7 cm dilated) at the time of call for transport. Five patients were delivered at the referring hospital and 49 were transported to a tertiary center in Phoenix. There were no deliveries en route and only 30% delivered in the first hour after arrival at the tertiary hospital. Transport of these patients was mostly by rotor wing aircraft (40) with 8 in fixed wing, and 1 ground transport. Almost 50% of these patients were 10 cm dilated at the time of call for transport. The Air Evac transport team consists of maternal flight nurses and neonatal flight nurses. These nurses have had a minimum of 2 years nursing experience in tertiary care L&D units and an extensive didactic course with an extended preceptorship. This study supports the concept that maternal transport can be accomplished despite advanced cervical dilatation. The experienced judgment of the maternal flight nurse is critical to these decisions to fly or not to fly.

466 QUADRUPLET PREGNANCY - CONTEMPORARY MANAGEMENT AND OUTCOME

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Quadruplet pregnancies are occurring more frequently as assisted reproductive techniques improve fertility in couples previously unable to conceive. Recommendation for selective reduction of quads to twins due to the "excessive" risk of quad pregnancies is not acceptable to all patients. This report describes 10 quadruplet pregnancies cared for in one perinatal practice over a 5 year period of time. This is the largest number of quad pregnancies ever reported from one center. These are compared to 57 consecutive quad pregnancies enrolled prospectively on home uterine activity monitoring nationwide by the Tokos Medical Corporation from October, 1986 to January, 1990. The mean gestational age at delivery for the Phoenix quads was 32.5 weeks compared to 30.2 weeks from the national quad group (sig p <0.001). All 40 babies in Phoenix survived without morbidity compared to a perinatal mortality rate of 127/1000 in the Tokos group (morbidity unknown). Of interest, 9 of 10 patients developed PIH in our series and 7 of 10 were delivered for worsening PIH, 2 of 10 for fetal distress, and only 1 of 10 for preterm labor. Maternal age does not impact outcome, but PMR for parous patients is lower than for nulliparas (p <.001). Our data reveals two gestational ages that are associated with an increased delivery rate: 29 to 30 weeks and unexpectedly 21-22 weeks. A team approach directed by an experienced perinatologist is vital to the improved outcome achieved in our series.

467 PRETERM PREMATURE RUPTURE OF MEMBRANES: IS OUTPATIENT MANAGEMENT APPROPRIATE?

D. Hoffman[X], G. Hansen[X], C. Ingardia, E. Philipson, Div Mat/Fet Med, Hartford Hospital, Hartford, Ct.

Preterm premature rupture of the membranes (PPROM) has traditionally been managed by prolonged hospitalization with delivery if infection or labor occur. For patients with PPROM who do not labor or demonstrate clinical signs of infection, outpatient management may be acceptable. The purpose of this study is to report the maternal and neonatal outcomes of patients with PPROM remote from term who, after 1 week of PPROM, remain undelivered and are followed as outpatients. Of 11,007 deliveries from January 1989 through April 1991, 82 patients (0.8%) with singleton pregnancies between 20-30 weeks were admitted with documented PPROM. Of the 21 patients (26%) who remained undelivered after 1 week, 12 (57%) were discharged and delivered at term. None of these patients had chorioamnionitis and all infants remained in the hospital for less than 4 days. One patient developed postpartum endometritis which was successfully treated with IV antibiotics. Nine patients (43%) delivered prematurely (range 26-34 weeks) and all reported increased leaking of amniotic fluid for days or hours prior to delivery. While 5 of these patients had clinical or laboratory evidence of infection, all neonates had five minute Apgar scores >7. Admission to the NICU for all the preterm neonates ranged from 12 to 72 days. In conclusion, the results of our study indicate that the majority of patients with PPROM will deliver within 1 week of admission. However, there appears to be a subset of patients with PPROM, (1 of every 7), characterized by the absence of labor or infection for 1 week, that may be managed as outpatients and deliver at term if adequate amniotic fluid volume is demonstrated by ultrasound and further leaking of amniotic fluid is minimal or absent.

468 FETAL LUNG MATURITY TESTING PROTOCOL: SURFACTANT/ALBUMIN RATIO. L.A. Bayer-Zwirello, B.A. Morris*, C.M. Kanaan, M.L. Gimovsky, J.P. O'Grady. Dept. of Ob/Gyn, Baystate Medical Center, Springfield, MA

An automated amniotic fluid surfactant-albumin ratio test (SAR) was performed for lung maturity on 137 pregnancies delivered within ≤3 days. 20/137 (15.6%) of neonates developed respiratory distress syndrome (RDS); 6/37 (4.9%) had transient tachypnea (TTN); the remaining 111/137 (78.8%) were clinically normal. The SAR had a sensitivity of 96.0%, a specificity of 75%; positive predictive value 46.1%; negative predictive value of 98.7%; interassay coefficient of variability 3.5%. Conclusion: The SAR for lung maturity is automated, rapid (≤ 1 hr), inexpensive, precise, and uses ≤ 1 cc fluid. Our protocol uses the SAR as our initial, rapid screening test for fetal lung maturity. The L/S ratio follows the SAR only if the initial study is immature. This protocol has reduced the number of L/S ratios required in our high risk population while retaining clinical accuracy and reducing cost.

469 NONFRANK BREECH PRESENTATION: EFFECT OF MANAGEMENT ON OUTCOME. D. Gauthier,[X] S. Warsof. University of Illinois at Chicago, Chicago, IL.

Nonfrank(NF) breech presentation is considered by many as an indication for C-section(CS). The purpose of this study was to assess the effect of intrapartum management on neonatal outcome. METHODS. Retrospective analysis of singleton NF breech deliveries ≥ 34 weeks EGA during a 10 year period was performed. Different managment plans included selective vaginal delivery(SVD) in which route of delivery was determined after evaluation of fetal weight, head position, and maternal pelvis, unselected vaginal delivery(UVD), elective CS for NF breech (ECS), and CS for other indication(OCS). RESULTS. Neonatal outcome for 290 NF deliveries is summarized as follows:

MANAGEMENT	SVD			UVD	ECS	OCS
	VD	CS-NTOL	CS-TOL			
NUMBER	86	53	14	24	95	18
5" APGAR <7	5(2)	0	1(0)	5(2)	6(2)	2(0)
BIRTH TRAUMA	1	2	0	1	2	0
MORTALITY	2(0)	0	1(0)	2(0)	2(0)	2(0)

VD=Vaginal delivery, CS-NTOL=CS-no trial of labor, CS-TOL=CS after trial of labor, ( )= corrected for congenital anomalies. CONCLUSIONS: (1) THERE WAS NO DIFFERENCE IN NEONATAL OUTCOME IN SVD VERSUS ECS, (2) CONGENITAL ANOMALIES WERE THE LEADING CAUSE OF MORTALITY.

470 ANTEPARTUM MANAGEMENT OF TRIPLET GESTATIONS. AM Peaceman, SL Dooley, RK Tamura, ML Socol. Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, Il.

Recent improvement in perinatal outcome for triplet gestations has been attributed to the use of routine antepartum hospitalization, home uterine contraction monitoring, tocolytic therapy, and cervical cerclage, but the value of these interventions has yet to be established. Furthermore, published series continue to report preterm delivery rates of 82-100%. We evaluated an alternative approach to the management of triplet gestations to determine its efficacy in the early diagnosis of preterm labor (PTL) and reduction of preterm birth. This approach included patient education regarding signs and symptoms of PTL, weekly prenatal visits with cervical examination after 24 weeks' gestation, and increased rest in an outpatient setting. Tocolytic therapy was restricted to gestations < 34 weeks in which progressive cervical change was documented in association with uterine contractions. Fifteen patients with triplet gestations were managed by this protocol over a 3 year period. Ten patients were hospitalized in the antepartum period for the following indications: PTL (4), advancing cervical dilation or effacement without contractions (5), and preeclampsia (1). Five patients received tocolytic therapy with $MgSO_4$, as one patient hospitalized for cervical dilation subsequently developed PTL; the interval from tocolysis to delivery was 37 ± 15 days (range 27-63). No patient was delivered because of failure to detect PTL in sufficient time to initiate successful tocolysis. The mean gestational age at delivery was 34.7 ± 2.6 weeks; 10 of 15 (67%) patients achieved 34 completed weeks' of gestation, and 6 (40%) completed 37 weeks. Indications for preterm delivery included labor at ≥ 34 weeks (4), premature rupture of membranes followed by labor (3), suspected placental abruption (1), and worsening preeclampsia (1). Mean birth weight was 1957 ± 488 grams, and 29 of 45 (64%) neonates did not require admission to the intensive care nursery. One neonatal death occurred secondary to necrotizing enterocolitis. We conclude that this management scheme for triplet gestations allowed for appropriate recognition of preterm labor and was as successful as protocols utilizing more expensive or invasive technologies in reducing the preterm delivery rate.

471 INCIDENCE OF MATURE L/S RATIO IN THE PRESENCE OF AN IMMATURE FOAM STABILITY INDEX (FSI). Asrat T, Towers CV, Lewis DL, Ogburn A[X], Nageotte MP, Women's Memorial Hospital, Long Beach, CA, University of California, Irvine, CA.

A commonly employed scheme of ascertaining fetal pulmonary maturity involves the use of the "maturity cascade" which consists of the "shake" test, followed by the FSI and finally the Lecithin Sphingomyelin (L/S) ratio if the first two tests indicate pulmonary immaturity. We conducted this study in order to determine the distribution of L/S values following an immature FSI, across various gestational ages. An immature FSI is defined as ≤0.46. From 1/86 to 12/89 857 samples of amniotic fluid were evaluated by an FSI. 259 of these samples had an FSI of <0.46 and 136 had an FSI equal to 0.46.

%Mature L/S (No.of Samples)

GA(Wks)	N	FSI≤0.46	N	FSI=0.46
28-29	4	0%(0)	1	0%(0)
29-30	7	0%(0)	2	0%(0)
30-31	12	0%(0)	4	0%(0)
31-32	25	0%(0)	8	12.5%(1)
32-33	45	4.4%(2)	18	5.5%(1)
33-34	36	8.3%(3)	26	30.7%(8)
34-35	59	8.4%(5)	32	28.7%(9)
35-36	42	7.1%(3)	24	46.0%(11)
36-37	29	31.0%(9)	21	62.0%(13)

%Mature L/S (95% CI)

GA(Wks)	FSI≤0.46	FSI=0.46	Total
<32	0/48	1/15	1/63-1.6%(0-4.6)
≥32	22/211	42/121	64/332 19.2%(15.0-23.5)

CONCLUSION: The above data indicate that at gestational ages below 32 weeks, if the FSI is ≤0.46 there appears to be no need to move on to an L/S. Furthermore, between 32 and 35 completed weeks, if the FSI is only <0.46 the rate of a mature L/S is less than 10%, and the routine assay for L/S may not be cost effective.

472 THE FAILURE OF ROUTINE AMNIOINFUSION IN PATIENTS WITH THICK MECONIUM TO ELIMINATE THE OCCURRENCE OF MECONIUM ASPIRATION SYNDROME. M.T. Parsons, A.K. Parsons,[X] and J.L. Angel. University of South Florida College of Medicine, Tampa, Florida.

The use of amnioinfusion in patients with thick meconium stained amniotic fluid (MSAF) to reduce complications of meconium to the fetus was first reported in 1988. The practice has become widespread but no data have been reported of a beneficial effect of routine use in a large patient population. We compared the incidence and complications of MSAF in 1987 (before amnioinfusion was used) to Jan.-Sept. 1990, (when amnioinfusion was routine for patients with thick meconium stained amniotic fluid). Patients' and babies' charts were reviewed for complications during labor, abnormal fetal heart rate tracings, use of amnioinfusion, and infant outcome, especially meconium aspiration syndrome. Results: The incidence of MSAF was 16.1% (1012 of 6275 deliveries) in 1987 compared to 16.9% (937 of 5537 deliveries) in 1990 (NS). Thirty-two infants were admitted to the NICU with meconium aspiration syndrome in 1987 which represented 3.2% of patients with MSAF, compared to 31 infants admitted to the NICU with meconium aspiration syndrome in 1990, 3.4% of patients with MSAF (NS). The mean stay in NICU in 1987 was 9.3 days and in 1990 8.8 days (NS). Of the 31 patients whose infants had meconium aspiration syndrome in 1990, only 6 had amnioinfusion performed and all 6 of these had abnormal fetal heart rate tracings. The reasons that the other 25 patients whose infants had meconium aspiration syndrome did not have amnioinfusion included: no suspicion of thick meconium (20), advanced labor (2), fetal distress necessitating delivery (2), and other (1). We conclude that routine amnioinfusion for suspected thick meconium does not eliminate meconium aspiration syndrome. Improvements may be made by 1) accurate and continued observation for the presence of thick meconium stained fluid, and 2) not falsely assuming amnioinfusion will always protect the fetus with thick meconium and an abnormal heart rate tracing.

473 CHANGES IN SUBSTANCE ABUSE OVER SUCCESSIVE PREGNANCIES: A LONGITUDINAL ANALYSIS. R.J. Sokol, S.S. Martier,[X] J.W. Ager, Dept. Ob/Gyn, Wayne State Univ./Hutzel Hosp., Detroit, MI

Though substance abuse is well recognized as a major pregnancy risk, there have been no longitudinal studies of maternal substance abuse in successive pregnancies. In this longitudinal study, prenatal risk factors were observed in two consecutive pregnancies for 388 black patients in an inner city prenatal clinic. Of particular interest were changes over time in substance abuse. For this sample, the age at the first pregnancy was 21.9 and mean inter-pregnancy interval was 23 months. Mean gestational age at first visit was about 22 weeks for both pregnancies. Differences between means for successive pregnancies were assessed using the matched t test for continuous measures and the McNemar significance of change test for the dichotomous outcomes in both cases using alpha = .01. Of the substance abuse factors, cigarette smoking showed a significant increase, ($p<.001$) whereas use of cocaine, cannabis and narcotics ($n=305$) showed no difference for the two pregnancies. For alcohol use, results depended on the period assessed. Periconceptional drinking, as measured by ounces of absolute alcohol per day (aad), amount per drinking day (aadd) and proportion drinking days (ppd), did not differ for the two pregnancies. However, for drinking at time of initial visit, the same three measures—aad, aadd and pdd—showed large and significant increases for the second pregnancy. A measure of alcohol-related life problems, the Michigan Alcohol Screening Test (MAST) score of ≥ 5 showed no differences between pregnancies (about 10% in each). Results reinforce the need for post-partum interventions designed to reduce maternal drinking before and during subsequent pregnancies.

474 PLACENTAL "LAKES" AND ANTICARDIOLIPIN ANTIBODIES (ACA). C. Hatjis, J. Graham[X], S. Wheeler[X], J. Gadd[X], K. Reed[X], Div. Maternal/Fetal Med., Dept. OB/GYN, Riverside Methodist Hospitals, Columbus, Ohio

ACA and lupus anticoagulant (LA) have been associated with significant obstetrical complications. Question: Does the sonographic detection of placental "lakes" correlate with the presence of ACA or LA in maternal blood? Methods: Placental lakes were seen in 23 pregnant women examined by ultrasound in the 2nd trimester. Transplacental lakes (TPL) spanned the entire width or a significant portion of the placenta and measured at least 2 cm in diameter. Subchorionic lakes (SCL) were located near the subchorionic plate. ACA and LA were measured by established methods. Results: 13 of 14 patients with TPL were positive for ACA (5/13 medium positive, 8/13 low positive); 1/13 was also positive for LA. In patients with SCL only 2/9 had low-positive ACA ($p < 0.001$). 10/14 patients with TPL, but only 3/9 with SCL had pregnancy related complications ($p < 0.06$). Conclusions: The presence of TPL strongly correlates with a positive maternal ACA panel and may serve as a marker for pregnancies at risk for complications. Intensive surveillance of patients with TPL and positive ACA is recommended.

475 PREVIOUS VERTICAL CESAREAN SECTION; UTERINE RUPTURE RATE

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Although the uterine rupture in patients undergoing a labor trial with a previous low transverse cesarean section is reported to be acceptably low, uterine rupture in patients with a previous vertical cesarean section is believed to be unacceptably high. However, studies of patients with previous vertical cesarean section labor trials have not represented all of these patients having a labor trial. The purpose of this study was to determine the frequency of clinically significant uterine ruptures in all baboons with previous vertical cesarean section. The reproductive histories of baboons from our colony between 1966 and 1991 were reviewed and those animals (n=29) who had undergone at least one labor trial after vertical cesarean section (VBAC) comprised the study group. A total of 188 deliveries occurred, 75 vertical cesarean sections, 104 VBAC (gestational age at least >100 days at delivery, term 175 days) and 9 uterine ruptures. The overall frequency of uterine rupture in these animals all having had a labor trial was 8.0% (9/113). The uterine rupture frequency increased with the number of previous vertical cesarean sections: one, 5.2% (3/58); two, 11.4% (4/35); three or more, 10.0% (2/20). The maternal mortality associated with uterine rupture was 38% (3/8) and the perinatal mortality 100%. Interestingly, the baboons in whom uterine rupture occurred tended to be older. Based on these experimental animal data, the frequency of uterine ruptures in nonhuman primates with a previous vertical scar having all had a labor trial is unacceptably high. Therefore, we would not recommend a labor trial for a patient with a previous vertical cesarean section.

476 **ELECTIVE CESAREAN HYSTERECTOMY IN RESIDENCY TRAINING.** M. Yancey,[X] F. Harlass, W. Benson, K. Brady, Madigan Army Medical Center, Tacoma, WA and William Beaumont Army Medical Center, El Paso, TX.

The utilization of elective cesarean hysterectomy (ECH) in select patients could eliminate the need for two seperate procedures and provide valuable resident experience. A survey of graduates from military residency programs demonstrated that 30% had no experience with cesarean hysterectomy during residency and only 45% had experience with peripartum hysterectomy as a primary surgeon. We studied the morbidity associated with ECH through a retrospective review of 45 patients that had undergone a scheduled procedure in a military training hospital between 1979 and 1989. We compared these findings to the combined morbidity of a scheduled cesarean delivery and subsequent abdominal hysterectomy in a control population of similar patients. Study patients were assigned two control patients matched for age, parity, number of previous cesarean deliveries, and indications for procedures. The number of women receiving transfusions following ECH was greater than the control population (39.5% vs 20.9% $P < 0.05$). However, the number of patients with major morbidity was significantly increased in the control population (44%) compared to patients with ECH (16%, $P < 0.01$). We conclude that patients undergoing ECH are less likely to have major perioperative morbidity, excluding the need for transfusion, than similar patients undergoing separate cesarean delivery and hysterectomy. Current residency training in elective or emergent peripartal hysterectomy is limited and could be increased through greater utilization of ECH.

477 **UMBILICAL ACID-BASE STATUS IN NEONATES AT HIGH ALTITUDE** M. Yancey[X], J. Moore[X], K. Brady, D. Milligan, W. Strampel[X], Evans Army Hospital, Ft. Carson, CO.

The analysis of umbilical cord blood acid-base status has proven useful in the immediate care and rescusitation of the newborn and provides an objective measure of the intrapartum fetal environment. The effect of an increase in altitude upon umbilical cord acid-base status has not been previously described. We studied the acid-base status of neonates delivered at a relatively high altitude of 6000 ft. (N=150) and infants delivered at 100 ft. (N=150). All patients had singleton pregnancies with an uncomplicated labor and vaginal delivery. Continuous electronic fetal monitoring was utilized and reviewed for evidence of impaired fetal perfusion. Exclusion criteria included fetal distress, hypertensive disease, diabetes, suspected intrauterine growth retardation, meconium stained amniotic fluid, or chorioamnionitis. Cord arterial and venous blood samples were collected and analyzed within 30 minutes of delivery. Statistical analysis was performed with the unpaired t test. Results are presented as mean values and * denotes $P<0.05$.

	pH	pCO_2	HCO_3	pO_2	O_2%
Venous					
6000 ft	7.392	33.4	20.2	29.2	57.5
100 ft	7.362*	37.8*	20.8*	28.0	54.8
Arterial					
6000 ft	7.322	44.7	22.9	18.1	27.0
100 ft	7.296*	46.3*	21.8*	17.3	26.3

We conclude that maternal adaptive measures to high altitude result in significant alterations in the umbilical blood acid-base status.

478 **THE FORCES CREATED BY THE OBSTETRIC BONNET ON THE FETAL HEAD: LABORATORY EVALUATION OF AN EXPERIMENTAL DELIVERY DEVICE.** B. Elliott,[X] L. Ridgway, E. Newton, M. Berkus, W. Peairs,[X] Univ. Texas Health Science Cen. at San Antonio, Texas.

The decline in the use of instrumental delivery is due, in part, to the concern regarding the forces vacuum extraction or forceps apply to the fetal head (30-60 cmHg vacuum and 5-20 lbs/in^2 compression, respectively). The obstetric bonnet is a disposable, dome-shaped, latex device. It resembles a large condom, and is rolled, placed on the fetal occiput and manually unrolled over the fetal head within the mother's vagina. Its elastic properties create an airtight seal on the fetal head, and a handle at its apex is used to manually apply traction. This study was undertaken to determine the forces this device creates on a laboratory model and to test its adherence during traction. A domed polypropylene cylinder with a 33cm circumference simulated the fetal head. An open port at the dome's apex measured the vacuum created and a closed bladder placed circumferencially around the cylinder measured lateral compression. Standard bourdon gauges were used to measure vacuum in cmHg and compression in lbs/in^2. A force gauge was attached to the handle of the device to measure the traction being experimentally applied. The devices were tested to a maximum traction force of 60 lbs in increments of 10 lbs. A significant relationship existed by simple regression between the traction applied and the vacuum and compression created as indicated below:

Traction Applied (lbs)	Vacuum (cmHg)*	Compression (lbs/in^2)**
10	10 ± 3	0.5 ± .3
20	18 ± 5	0.6 ± .3
30	23 ± 5	0.7 ± .3
40	27 ± 5	0.9 ± .4
50	31 ± 6	1.1 ± .5
60	33 ± 7	1.2 ± .3

n = 18 Mean ± SD *r^2 = .85, $p < .00001$ **r^2 = .42, $p < .0004$

The data from this model indicate that this device: 1) provides adequate adherence to allow traction forces commonly used in instrumental delivery; 2) creates vacuum and compression forces that compare favorably to those created by other instruments; and 3) creates graded vacuum and compression determined by the traction applied. This is an encouraging preliminary evaluation of this new device.

479 PERIMORTEM CESAREAN SECTION IN MICHIGAN: "ARE WE GETTING BETTER?" Chang Y. Lee, M.D., Elaine M. Mills,[X] Maternal Mortality Committee, Michigan State Medical Society, Center for Health Promotion, Michigan Department of Public Health, Lansing, Michigan.

Eight (25%) infants survived from 32 perimortem Cesarean sections performed for 15 years, from 1972 to 1986 in the State of Michigan. The overall survival rate was not significantly improved compared to that of postmortem sections performed from 1950 to 1957 (25% vs. 15.3% $P < 0.05$). In 19 cases, the time of delivery and maternal death was recorded. No infants survived from six cases when the procedure was performed after maternal death. Six (46%) infants survived from 13 cases when the procedure was performed before death of the mother. This is clearly less than the 70% survival rate when the procedure was performed within five minutes after maternal death in reported series. It is apparent from the study that the procedure should be performed before the death of the mother to ensure the best chance of the infant's survival.

480 **ADOLESCENT PREGNANCY OUTCOME.** J.L. Davis, A. Pisani,[X] B.R. Morgan,[X] F. Wall,[X] J Greenspoon, Cedars-Sinai Med Ctr. LA, CA.

To determine whether maternal age influenced obstetrical outcome, we compared a group of young adolescents (age ≤ 17 yrs) to a group of older women (ages 21-24 yrs). All were patients of a health maintenance organization (HMO) who delivered between 1985 and 1990. The mean (SD) age was 16.1 (0.97) for adolescent and 22.6 (1.1) for the older group. Black race was more common among adolescent (63.1%) than the elder group (48.1%). Logistic regression was used to examine the relationship of obstetrical outcome variables to race and subsequently to maternal age. After accounting for race, we found that adolescent pregnancies differed statistically but not clinically from those of the older women with respect to birthweight (Table). The adolescents were 2.5 times more likely to develop preeclampsia than the older women ($p < 0.001$). Conversely, older women were twice as likely to develop gestational diabetes ($p < 0.05$) as the adolescents. During the time interval studied, 59 (7.5%) of the adolescents and 282 (8.8%) of the older group delivered a second pregnancy. The time interval between pregnancies did not differ (Table). Young maternal age had a limited and predictable effect on obstetrical outcome in women receiving care through a HMO.

Table

	Adolescents N=786	Control N=3198	P*
Gest age (wks)	39.2±2.53	39.4±2.34	NS
Birthwt. (gms)	3175±557	3290±564	<0.001
5 min Apgar	9±1	9±1	NS
Cesarean delivery	17%	18.8%	NS
Preterm delivery	13%	9.3%	0.0079
Posterm delivery	21%	21%	NS
Interval between preg (days)	632±660	568±229	0.0620

*NS= not significant

Values are means ± standard deviations or percentages.

481 **SUBCUTANEOUS TISSUE: TO CLOSE OR NOT TO CLOSE AT CESAREAN SECTION** V.R. Bohman MD[X], L. Gilstrap MD, K. Leveno MD, S. Ramin MD[X], R. Santos-Ramos MD[X], K. Goldaber MD[X], B. Little PHD[X], J. Dax RN[X]. University of Texas Southwestern Medical Center, Dallas, Texas.

There is no unanimity of opinion regarding suture closure of Scarpa's and Camper's fascia at time of c-section. Over a 4 month period, all cesarean section patients had their subcutaneous tissues sutured closed with interrupted 000 plain cat gut suture or left open on alternating months. Suspected risk factors of subsequent wound separation were rigorously sought in each patient. This study included 871 patients, 457 with subcutaneous tissues closed and 414 left open. Labor was present in 57.5%, 13.7% had ruptured membranes for >24 hours, 7.3% had amnionitis, and 4.5% had diabetes. These factors failed to differ in these two groups. At c-section, 85% had a vertical skin incision, 11.6% of the cases took >1 hour to complete and 4.9% had a decrease in hematocrit by 10 percentage points. The overall wound separation rate was 7.2% (62 of 871), 6.6% (30 of 457) of the suture closed group and 7.7% (32 of 414) of the non-closed group ($P = 0.504$; $OR = 0.838$). Culture proven wound infections were present in 23 (2.6%) women and 2 (0.2%) had a fascial dehiscence. We were unable to identify any risk factors for wound separation including whether the subcutaneous tissues were sutured together or not in this study population. Our data suggests that the surgeon's personal preference is appropriate to determine whether the subcutaneous tissues should be closed with suture or not.

482 **STANDARDS FOR FETAL GROWTH IN ADVANCED MATERNAL AGE PATIENTS.** J. Williams III, B.T. Wang[X], R. Willis-Hassan[X], The Prenatal Diagnostic Ctr. of S. CA, Beverly Hills, CA

Published standards for fetal growth are derived from general patient populations (GP) which consist of patients of all maternal ages but are skewed toward younger patients. We hypothesize that infants delivered to advanced maternal age (AMA) patients have larger birthweights (BW) than infants delivered to GP patients and that published BW standards may not be appropriate for use with AMA patients. To test this hypothesis, BW and delivery data were obtained from 2473 liveborn, singleton, cytogenetically normal infants delivered to AMA (age ≥ 35 at EDC) patients. Gestational age (GA) at delivery was based on completed weeks (WKS) from LMP. All GA's were confirmed by a 1st or 2nd trimester sonogram. The racial distribution was 86% white and 14% non-white. The 10th, 50th and 90th percentiles were calculated for each GA and compared with published standards for singleton births near sea level (Brenner, Williams). The BW in our series were comparable at GA < 35 WKS and were consistently greater at all percentile ranks than published standards at GA ≥ 35 WKS. This suggests that BW curves derived from AMA patients should be used to evaluate fetal growth in AMA patients.

483 **THE CERVICAL CAP DOES NOT PREVENT PULMONARY HYPOPLASIA IN SECOND TRIMESTER RUPTURE OF MEMBRANES.** B.V. Parilla, R.K. Tamura, M.L. Socol, Division of Maternal-Fetal Medicine, Northwestern University Medical School, Chicago, Il

Premature rupture of the membranes (PROM) is one of the most common causes of preterm delivery and neonatal morbidity. Particularly in the second trimester, PROM is associated with a high incidence of pulmonary hypoplasia, compression deformities, and neonatal death. Recent evidence suggests that residual amniotic fluid volume may be an important factor in affecting outcome. To test the hypothesis that reaccumulation of amniotic fluid would improve perinatal outcome, we have applied a cervical cap as part of an ongoing study in 2 pregnancies complicated by PROM <23 weeks with oligohydramnios to recreate a seal and thereby reaccumulate amniotic fluid. The first cap was placed at 21 4/7 weeks' gestation, 72 hours after ROM (Amniotic fluid index (AFI):0). Amniotic fluid reaccumulated within 48 hours. The patient remained stable for 4 weeks with a mean AFI of 9.6 cm (range 5-10). At 25 6/7 weeks' gestation, a low lying placenta with bleeding necessitated removal of the cap and this was followed by the immediate loss of all fluid. Labor ensued within 2 days and a neonate with pulmonary hypoplasia (birth weight 1015 gm, lung weight 14.8 gm) was delivered and expired. The second cap was placed at 20 1/7 weeks' gestation, 84 hours after ROM (AFI 0). Again, amniotic fluid reaccumulated over 48 hours. The patient remained stable for 7 weeks with a mean AFI of 5.0 cm (range 3-6). At 27 4/7 weeks spontaneous labor ensued and again the neonate had pulmonary hypoplasia and expired (weight 1013 gm, autopsy denied). These observations disappointingly suggest that the reaccumulation of amniotic fluid after PROM may not prevent pulmonary hypoplasia.

484 EFFECT OF ANGULAR TRACTION ON THE PERFORMANCE OF MODERN VACUUM EXTRACTORS, Kevin Muise, M.D.· Method A. Duchon, M.D., Richard H. Brown, Ph.D.[X], Departments of Repro. Biol., University MacDonald Womens Hospital and Surgical Research St. Luke's Hospital, Cleveland, Ohio.

A baffling array of vacuum extractors exists for operative vaginal delivery. The purpose of the present study was to describe the effect of off-axis traction on the performance of modern vacuum extractors. Eight vacuum extractors were examined in the laboratory using a force indicator and fetal cephalic model. Devices evaluated included the 6 cm. Malmstrom, Mity-Vac, M-Type, O'Neil, 5 cm. Posterior, Silc, Tender-Touch, and Silastic. Maximal tractive (pop-off) force was measured for each device in increments of ten degrees from the vertical. Results revealed the best fitting model to be Force = Constant+Angle+Vacuum. Each device was described by a unique regression plane, where the regression coefficient for angle was negative in all devices except the Posterior cup. At increasing angles of traction, maximal force decreased in the following order: Silastic, Silc, Tender-Tough· M-Type, Mity-Vac. O'Neil, Malmstrom, and Posterior. These results suggest that an understanding of performance may allow tailoring of cup selection to the clinical situation.

485 **THE SIGNIFICANCE OF FETAL HEART RATE (FHR) BASELINE IN THE POSTDATES PATIENT.** D.N. Platek[X], M.I.G Morel[X], A.D. Marks[X], H.W. Hsu[X], M.Y. Divon. Dept. Ob/Gyn, Albert Einstein College of Medicine, Bronx, N.Y.

It has been noted that the resting FHR baseline in some postdates patients is lower than that previously defined as normal (120-160 bpm). The purpose of this study was duo-fold: (1) To determine the normal range of FHR baseline in the postdates patient, (2) To evaluate the association between FHR baseline and outcome in the postdates patient. The study population consisted of 196 consecutive patients evaluated by non-stress testing who met the following entry criteria: no medical or obstetrical complications other than being postdates, certain EDC based on LMP and confirmed by early ultrasound and a minimum of two postdates NSTs. Intrapartum FHR tracings and outcome data was also reviewed for these patients. Adverse outcome was defined as the presence of thick meconium, 5 minute apgar score <7, fetal acidosis (scalp or cord artery pH <7.2), or NICU admission. The mean gestational age at the first postdates visit was 41.2 ± .4 weeks (mean ± S.D.), with a mean FHR baseline of 133.9 ± 9.3 bpm and a range of 110-160 bpm. The gestational age at last visit was 42.1 ± .5 weeks, with a mean FHR baseline of 133.2 ± 9.4 bpm and a range of 110-158 bpm. 10% of patients had a resting FHR baseline of 120 bpm or lower on non-stress testing. Longitudinal trends of FHR baseline were evaluated by Wilcoxon and standard regression analyses and revealed no significant decrease in FHR baseline as a function of advancing gestational age. In addition, there was no relation between either antepartum or intrapartum FHR baseline <120 or >150, and adverse fetal outcome in this patient population. In conclusion, the range of antepartum FHR baseline in our postdates patient was found to be 110-160 bpm with a mean of 133 bpm and a FHR baseline within this range is associated with favorable outcome.

486 PROPHYLACTIC CERCLAGE IN TRIPLET PREGNANCY.

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Prophylactic cerclage was performed in 12 triplet pregnancies. Pregnancy outcomes were compared to 15 triplet pregnancies managed without cerclage placement. All gestational dates were confirmed by knowledge of date of conception by assisted reproduction technology or by first trimester ultrasound measurement. All cerclages were performed at 11 to 15 weeks and no complications occurred. Home uterine activity monitoring, tocolysis, bedrest, and hospital admission were utilized as needed in both groups. Results:

	Cerclage		No Cerclage
Mean Gest Age (wks)	34.8		33.5
Range	32.1 - 36.3		27.7 - 38.2
Mean Birthwt (gms)	2081		1899
Range	1525 - 2770		910 - 3020
Mean # NICU Days	8.4	p<.02	18.8
Range	0 - 23		0 - 85

Prophylactic cerclage placement in triplet pregnancy was associated with a significant reduction is length of stay in the neonatal ICU and a prolongation of gestation by 10 days. No incidences of infants less than 1500 grams or less than 32 weeks gestation occurred in the cerclage group. Cerclage placement may allow more consistent outcome in triplet gestations.

487 **TRIAL OF LABOR AFTER A ONE OR TWO LAYER CLOSURE OF A LOW TRANSVERSE UTERINE INCISION**

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There is little documentation of the safety of a subsequent trial of labor following a one or two layer uterine incision closure. We reviewed the charts of 258 women who had a low transverse cesarean section with no vertical extensions and who had a subsequent labor and either a vaginal or cesarean delivery in our unit. In 123 women the uterine incision was closed in one continuous layer of a locking #1 chromic gut suture and in 135 it was closed in two continuous layers of #1 chromic gut with the first layer locked. Maternal morbidity, specifically endometritis, post partum blood loss, intra-operative complications, post-operative atelectasis and ileus were similar in both groups during the subsequent pregnancy and delivery. Three women in each group had an asymptomatic scar separation confirmed at repeat c-section. No symptomatic uterine rupture or adverse perinatal outcome occurred in these 258 women. We conclude that closure of a lower uterine transverse incision in one continuous layer should not preclude a trial of labor after one prior cesarean section.

488 **DOES NONCLOSURE OF BLADDER FLAP AT CESAREAN SECTION DECREASE FLUID COLLECTION AND INFECTIOUS MORBIDITY?** J.D. Jacobson[X], G.N. Gregerson[X], G.J. Valenzuela. Department Obstet Gynecol, San Bernardino County Medical Center, San Bernardino, CA.

The re-approximation of visceral peritoneum over the lower uterine segment at cesarean section has been shown to be associated with significant bladder flap fluid collections in up to 30% of patients studied, and has also been shown to be a site of pelvic abscess. We hypothesized that nonclosure of peritoneum would decrease fluid collection and decrease infectious morbidity. To study this question, we randomized a group of 61 patients to have either the bladder flap closed, or left open. In all patients parietal peritoneum was not sutured and prophylactic antibiotics were used. An ultrasound was done on the third post-op day to assess bladder flap fluid collection. A data base was prospectively collected on all study patients. 30 patients were randomized to the "closed" group, and 31 patients to the "open" group. Outcomes, including fluid collection, paraendometritis, and post-operative fever were similar between the two groups. Nonclosure of the bladder flap at cesarean section is not associated with decreased fluid collection or infectious morbidity.

489 ANTEPARTUM HOME CARE FOR HIGH-RISK PATIENTS: AN ALTERNATIVE TO HOSPITALIZATION R. Kempfer-Kline,[X] T.Lubart Spector,[X] R.J. Wapner, G.H. Davis, Dept. of Ob/Gyn, Jefferson Medical College of Thomas Jefferson Univ. Hosp., Philadelphia, PA

The management of high-risk pregnancies frequently involves bedrest and prolonged costly hospitalization which separates the woman from her environment. We evaluated the hypothesis that high-risk pregnancy management could be safely provided at home. Materials and Methods: From 1/1/90 - 7/1/91, 80 high-risk pregnant women (102 fetuses: 8 triplets, 6 twins) were managed by a home high-risk management protocol. Patients were admitted following either an antepartum hospitalization (64%) or from the physician's office (36%). In lieu of prolonged hospitalization, patients had, at least, 1 home visit per week by a nurse trained in perinatal care. Visits included FHT's, measurement of fundal height, urine dip stick, cervical examination, NST's, limited ultrasound, childbirth and nutritional counseling, phlebotomy, and subQ or IV fluids. NST's were faxed for evaluation by the perinatal physician. Services were performed by a nurse with the exception of ultrasound, which was provided by a home visit from an MFM specialist. Patients were readmitted to the hospital for acute changes in their condition. Results: Program patients included: preterm labor 58%; incompetent cervix 16%; hypertensive disease 7.5%; antepartum hemorrhage 5%; intrauterine growth retardation 4%; PROM 4%; polyhydramnios 2.5%; other 3%. Thirteen (16%) required, at least, one further antepartum admission and 35 (44%) were admitted to the hospital for a change in their condition or delivery requiring discharge from home care services. Of the 76 completed pregnancies there were 4 losses: 2 from PROM <22 weeks; 2 from twin-twin transfusion. Thirty-seven (49%) delivered at $\geq$ 37 weeks; 28 (37%) delivered 33-36 weeks; 8 (10%) between 28-32 weeks, and 3 (4%) $\leq$ 27 weeks. These statistics compare favorably with patients managed in hospital. Conclusion: High-risk pregnant women can be safely managed at home in lieu of prolonged antepartum admission.

490 **COMPARISON OF POSTPARTUM PAIN AND HEALING WITH REPAIR OF PERINEAL DISRUPTIONS USING CHROMIC CATGUT OR POLYGLYCOLIC ACID SUTURE.** C.L. Wikoff MD[X], T.J. Kuehl PhD[X], A.T. Cooney RN[X], A.B. Knight MD, Scott & White Memorial Hospital & Clinic, Texas A&M University College of Medicine, Temple, Texas.

Studies, primarily in the English literature, suggest that repair of perineal disruptions is less painful if accomplished with polyglycolic acid (PA) rather than chromic catgut (CG) suture. In this study 431 patients were prospectively randomized to repair with PA or CG. 345 required repair. Postpartum pain was assessed by the ACCS (Analog Chromatic Continuous Scale, range 0 to 100 mm) and amount of prn medications requested. Perineal healing was evaluated by the REEDA scale (redness, edema, ecchymosis, discharge and skin approximation; each characteristic score was 0-3 and total scores 0-15). Perineal disruptions were divided into 3 groups: no episiotomy or laceration (I), 1st and 2nd degree (II), and 3rd and 4th degree disruptions (III).

Group	N	ACCS (x±SE)	REEDA (x±SE)	Comps
I	86	9.9 + 1.5	0.80 + 0.14	0
II+CG	125	17.3 + 1.6	1.51 + 0.22	1
II+PA	149	16.5 + 1.4	1.40 + 0.15	1
III+CG	42	29.6 + 3.5	3.34 + 0.45	1
III+PA	29	33.5 + 3.7	2.91 + 0.35	0

Pain and perineal appearance increased significantly with extent of disruption but not with suture type. No differences in pain medications nor length of hospital stay were found between groups or sutures.

491 **A PRACTICAL AND COST EFFICIENT APPROACH TO UMBILICAL ARTERY pH AND BLOOD GAS DETERMINATIONS.** Norman B. Duerbeck,* David G. Chaffin,* John W. Seeds, Arizona Health Sciences Center, Tucson, Arizona

The purpose of this study was to assess a simplified method of umbilical artery pH sampling and to determine the effect of delay of sampling upon umbilical artery pH, pCO_2, and pO_2. Twenty-five umbilical cords were sampled at time of delivery and every fifteen minutes thereafter up to one hour postpartum from umbilical cord segments left at room temperature. The blood samples were collected in non-heparinized and non-iced plastic syringes. Sixty minutes after delivery, the average change in pH was 0.01 (range 0-0.08). None of the changes in pH, pCO2, or pO2 were statistically significant by paired Students's t test ($p>0.05$). Further analysis of pH data using multivariate analysis of variance (MANOVA) demonstrated no significant change up to one hour after delivery ($p=0.698$). Our results indicate that umbilical artery blood gas determinations can be obtained from umbilical cords left at room temperature for up to one hour after delivery and collected with non-heparinized, non-iced syringes without a significant change in pH.

492 THE USE OF THE NONSTRESS TEST AND THE FETAL BIOPHYSICAL PROFILE IN THE EXPECTANT MANAGEMENT OF PATIENTS WITH PROLONGED PRETERM PREMATURE RUPTURE OF THE MEMBRANES.
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The role of the nonstress test and the fetal biophysical profile in the management of prolonged preterm PROM was evaluated in 68 consecutive patients who had a latency period of more than 48 hours. Fetal surveillance consisted of daily nonstress tests and biophysical profiles every two to three days. Patients were delivered due to spontaneous labor, clinical chorioamnionitis, fetal distress, or upon reaching 37 weeks gestation. We found a statistically significant association between an abnormal nonstress test and overall infectious complications (chorioamnionitis plus neonatal infections), neonatal infections (sepsis and pneumonia), and fetal distress. A biophysical profile score ≤ 6 was associated with fetal distress, as was the combination of absent fetal breathing movements and nonreactive nonstress test. The association between a low biophysical profile (≤ 6) or the combination of absent fetal breathing movements plus a nonreactive NST and the development of chorioamnionitis or neonatal infectious complications did not reach statistical significance. This study suggests a role for the use of the NST in the management of patients with prolonged preterm PROM, but is less encouraging in terms of defining a role for the biophysical profile as the main tool for fetal surveillance in cases of prolonged preterm PROM.

493 **NORMAL MATERNAL BODY MASS INDEX IS ASSOCIATED WITH GOOD PERINATAL OUTCOME IN POSTDATES PATIENTS.**
C. O'Reilly-Green[x], M. Divon, Albert Einstein College of Medicine, Bronx, NY.

Body mass index (BMI), defined as weight divided by height squared, is used as a measure of obesity. Various recommendations have been made regarding the optimal pregnancy weight gain. However, paucity of data exists regarding the optimal BMI in pregnancy. Purpose: To evaluate the association between pregnancy outcome in postdates patients and maternal BMI. Postdates patients are interesting in this regard because IUGR and macrosomia can be studied after the exclusion of hypertension and diabetes. The BMI was evaluated prospectively in 158 postdates patients (over 41 weeks by accurate dates). An adjusted BMI (aBMI) was calculated by subtracting the maternal mass attributable to pregnancy from the calculated BMI and was correlated with the incidence of C/S, macrosomia, small for gestational age (SGA), oligohydramnios, low Apgar score at 5 minutes, acidosis and NICU admission. A normal aBMI was defined as less than 23.5 Kg/m^2. Results:

	Normal aBMI	High aBMI	p value
Cesarean section	1/27	53/131	.0006
Macrosomia	0/27	26/131	.03
Small for gestational age (wt <2800gm)	2/27	5/131	NS
Oligohydramnios	5/24	32/127	NS
Low Apgar or pH	0/24	16/127	NS
NICU admission	0/24	5/127	NS

In conclusion, normal maternal weight for height (aBMI) in postdates pregnancy is associated with a lower incidence of cesarean section and macrosomia. Normal aBMI is not associated with increased perinatal morbidity despite the fact that this group contains individuals who may actually be underweight.

494 **DOES UNEXPLAINED SECOND-TRIMESTER MATERNAL SERUM HUMAN CHORIONIC GONADOTROPIN ELEVATION PREDICT PERINATAL COMPLICATIONS?**
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This cohort analytic study was undertaken in order to examine whether women with unexplained human chorionic gonadotropin (HCG) elevation at 16 to 20 weeks gestation are at increased risk for perinatal complications as has been shown for women with unexplained elevation of maternal serum alpha-feto protein (MSAFP). We searched the data base of our laboratory for all cases of unexplained HCG levels > 2.5 MOM (with normal MSAFP) during the year 1990. We then assessed the delivery records of these patients for various maternal and perinatal complications and compared them to a group of randomly selected controls whose HCG as well as MSAFP were normal. Delivery records were available for 262 (91%) patients with elevated HCG and 265 (92%) of the controls. Elevated HCG was found to be associated with a significantly increased risk for IUGR - odds ratio 2.75 (95% CI 1.08 - 7.02) and for hypertension - odds ratio 4.35 (95% CI 1.88 - 10.06). The risk for the various perinatal complications was computed with multiple logistic regression to adjust for the effects of risk factors such as maternal age and obstetrical history. The risks associated with high HCG were unchanged by adjustment for these factors. We conclude that women with unexplained elevated HCG are at increased risk for hypertension and IUGR.

495 AMNIOTIC FLUID INDEX (AFI) AS A PREDICTOR OF LATENCY AFTER PRE-TERM PROM
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Patient records were reviewed to identify patients admitted with a diagnosis of pre-term PROM. 51 patients with initial evaluation within 48 hours of rupture and gestational age 26-34 weeks were identified. AFI was evaluated along with a Biophysical Profile, these were followed serially until delivery. Delivery was accomplished because of spontaneous labor, chorioamnionitis or fetal compromise. Tocolytics were not used. AFI at initial evaluation was stratified into 3 groups: Low (AFI<5), Reduced (5.0-7.9) and Normal (≥8.0). Latency in days from PROM to delivery was evaluated for these groups. A significant ($P<0.01$) difference was noted using the Kruskal-Wallis test. Wilcoxon two-sample (Rank-Sum) test scores showed that latency was significantly longer in the group with Normal AFI vs Low ($P<0.005$) or Reduced ($P<0.05$) AFI groups. This knowledge should allow better patient selection for various intervention strategies, such as corticosteroid administration or antibiotic amnioinfusion. This striking result makes the AFI a powerful tool for predicting latency after PROM.

496 ANTENATAL TESTING USING THE AMNIOTIC FLUID INDEX (AFI) BEYOND 280 DAYS GESTATION

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500 patients were referred for antenatal testing beyond 280 days EGA. AFI and Biophysical Profiles (BPP) were evaluated twice weekly. Adverse labor events and neonatal outcomes were analyzed according to the AFI using Receiver Operating Characteristic (ROC) analyses. Spontaneous and induced labors were included. Clinicians were not blinded to the AFI or BPP scores. The ROC curve for all EGAs shows only a modest effect for AFIs<12.5, above this true positives and false positives are equal. Below this level the ROC curve is quite shallow indicating minimal effectiveness. From 281-287 days the ROC curve indicates better predictive ability; an AFI cur-off of 9.0 yields sensitivity of 100% and specificity of 57%. At more advanced gestational ages the utility reverts to that seen overall; for example and AFI cutoff of 9.0 yields 44% sensitivity and 65% specificity. This lack of prediction of pathology mirrors the known downward trend of AFI in normal pregnancies beyond 40 weeks and makes it imperative to interpret "oligohydramnios" cautiously at advanced gestational age. AFI norms at greater EGAs are needed, but we have shown that between 281-287 days an AFI<9.0 is a predictor of adverse events or outcomes.

497 OLIGOHYDRAMNIOS FOLLOWING PROM; IS THE AMNIOTIC FLUID INDEX (AFI) PREDICTIVE OF OUTCOME?

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To test whether oligohydramnios following PROM is predictive of adverse labor or neonatal outcomes the records of 193 patients serially tested in the Fetal Diagnostic Unit were reviewed. The last AFI before delivery was subjected to Receiver Operating Characteristic (ROC) analyses. Patients either labored spontaneously or were delivered for indications other than oligohydramnios. Patients had been managed expectantly without tocolytics. AFI was virtually non-predictive at delivery≥36 weeks; the ROC curve was flat with true positives and false positives essentially equal. For patients with EGA at delivery <36 weeks the ROC curve shows modest prediction of adverse outcomes (other than those related simply to prematurity). Two operating points are suggested by the shape of the ROC curve; a strict cut-off of AFI <4.0 gives sensitivity of 64% and specificity of 72%, a liberal cut-off of AFI <6.0 gives sensitivity of 84% but only a specificity of 43%. Thus AFI is not useful in near-term PROM but should influence the decision of whether to intervene or manage pre-term PROM expectantly. Oligohydramnios <36 weeks may reflect placental insufficiency along with mechanical leakage.

498 FETAL HEART RATE MONITORING FOLLOWING ANTENATAL BLEEDING.

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Antepartum bleeding is associated with fetal stress and increased perinatal morbidity and mortality. It is proposed that as a result of the stress, central nervous system maturation will be accelerated in the fetus. It is assumed that bleeding will result in specific fetal heart rate (FHR) changes reflecting this process (sympathetic). Our hypothesis was that early antenatal bleeding will result in accelerated sympathetic maturation expressed by matured FHR patterns in the preterm infant. 91 patients with severe antenatal bleeding and 91 controls with uncomplicated normal pregnancies participated in the study. All women were not in labor and were hospitalized in the High-Risk Pregnancy Unit at 24-37 gestational weeks. Excluded were patients with premature rupture of membranes, intrauterine growth retardation, diabetes, hypertension and twins. FHR tracings were analyzed during the first week from the initial episode of bleeding and compared to the control group. FHR tracings were analyzed for basal heart rate, long-term variability, number and amplitude of accelerations in the best 20 min segment.

Gest. Age	*24-29 wks*		*30-33 wks*		*34-37 wks*	
Group	*Bleeding*	*Control*	*Bleeding*	*Control*	*Bleeding*	*Control*
Mean GA	27.6	27.2	31.4	31.3	35.9	35.4
Baseline HR	142.5	142.7	139.4	140.7	136.7	137.4
# Accelerations in 20 min	4.4**	1.0**	5.5**	3.9**	7.7**	5.3**
Amplitude of acceleration	20.7**	18.5**	21.7**	18.7**	22.7*	21.6*
% of reactive monitors	89**	37**	95**	71**	100**	87**

*p<0.05 **p<0.001

The study also revealed: 1) both placenta previa and abruptio placenta had significantly larger numbers of accelerations with a higher acceleration amplitude when compared to the control group; 2) no significant differences were found in the FHR characteristics between these two causes of antenatal bleeding. Our data suggest that accelerated sympathetic maturation expressed by reactive FHR tracing is prominent in pregnancies complicated by antenatal bleeding due to abruptio placenta and placenta previa.

499 LONGITUDINAL AMNIOTIC FLUID INDEX IN POSTDATES PREGNANCIES AND ITS ASSOCIATION WITH FETAL OUTCOME.

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Due to the known association between oligohydramnios and adverse fetal outcome, sonographic evaluation of amniotic fluid index (AFI) is extensively used for fetal testing in postdates pregnancies. However, the relationship between dynamic changes in AFI and fetal outcome is unknown. Purpose: To study the dynamic changes in AFI measurements and their association with adverse fetal outcome in postdates pregnancies. Serial AFIs were obtained semi-weekly in 139 reliably dated (certain LMP consistent with early sonographic exam) pregnancies > 41 weeks' gestation. Each patient was evaluated on 2 - 6 separate occasions and a total of 331 tests were performed. Indications for delivery included poor fetal testing, oligohydramnios (i.e. AFI≤5.0cm) or a favorable cervix. Adverse fetal outcome was defined by the presence of moderate or thick meconium, FHR decelerations, C/S for fetal distress or NICU admission. The AFI increased in 43 patients (a mean increase of 2.5 cm ± 2.7, ±SD), did not change in 2 patients and decreased in 96 patients (a mean decrease of 3.7 cm ± 2.7). Fourteen of these patients had a final AFI ≤ 5.0cm. Prominent changes in AFI (i.e.± 66%) had no association with adverse fetal outcome. A significant association with the outcome variables was only detected in patients whose final AFI was ≤5.0 cm (p=0.00001). Mean birth weight was significantly lower in those patients who had a final AFI ≤ 5.0cm. The mean gestational age for patients with a normal AFI was 42 weeks + 3 days and for those with oligohydramnios was 42 weeks + 1 day (p=NS). Neither birth weight nor gestational age were predictive of fetal outcome. There was no significant association between oligohydramnios and gestational age. Thus, there was a significant increase in adverse fetal outcome when the final AFI ≤ 5.0cm, irrespective of the rate of change in AFI. Fetal outcome was not predictably associated with either gestational age or birth weight in these patients.

500 **A LONGITUDINAL STUDY EVALUATING THE EFFECT OF GESTATIONAL AGE ON ANTENATAL ASSESSMENT TESTS.** S.J. Carlan, M. Gore*, S. VanMeter*, D Mastrogiannis,U of S FL,Depts Ob/Gyn Tampa,FL, ORMC,Orlando,FL

The purpose of this study was to evaluate the effect of gestational age on antenatal assessment tests using the same group of patients throughout their pregnancy. Fourteen normal pregnant women with normal fetuses were studied every two weeks from 20 wks until delivery. Gestational ages were confirmed by early exam and ultrasound. A biophysical profile (BPP) and non-stress test (NST) were performed in the standard manner at each visit. Umbilical artery S/D ratios (S/D) were obtained using a continuous wave doppler and were recorded during fetal apnea. A startle reflex was considered positive if a quiet fetus was demonstrated to startle on real time ultrasound within one second of a three second application of sound using the standard artificial larynx. A vibrocoustic stimulation (VAS) was considered positive if a similar sound challenge resulted in a >15 beat per minute increase in fetal heart rate for >15 seconds. All maternal and neonatal outcomes were normal. The mean birth weight was 3569 ± 169 grams.

WKS	BPP	SD	NST	VAS	STARTLE
	%>6	Mean ±1SD	(% reac)	(% reac)	(%+)
20	21	4.1 ± .7	0	0	0
22	14	4.0 ± .7	0	14	0
24	36	3.8 ± 1.0	7	14	0
26	86	3.4 ± .7	57	29	0
28	92	3.5 ± .8	62	92	85
30	91	3.2 ± .8	85	85	92
32	100	2.8 ± .8	100	92	92
34	92	2.7 ± .5	85	85	85
36	100	2.6 ± .4	85	92	92
38	78	2.3 ± .4	88	100	100
40	75	2.3 ± .5	67	100	66

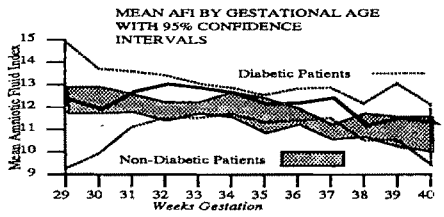
We conclude that, like previously reported cross-sectional studies, antenatal surveillance is highly gestational age dependent, and in fetuses less than 26 wks associated with a high false non-reassuring rate.

501 **WHAT IS THE INTERVENTION RATE IN PATIENTS IN ANTEPARTUM TESTING FOR A PRIOR STILLBORN?** Dena Towner MD*, Richard Paul MD University of Southern California Los Angeles, California

In the era prior to antepartum evaluation and intervention for fetal indications, the Collaborative Perinatal Study published in 1972 that the perinatal mortality was 73 per 1000 in the pregnancy subsequent to a stillborn(SB). In contrast, our current institutional SB rate in women undergoing antepartum testing is 2/1000. The women at greatest risk of repeat SB are those with another known risk factor; ie, hypertension, diabetes and current IUGR. However this leaves a significant population with no other known risk factors. The purpose of this study is to determine if a significant number of antepartum tests (APT) led to intervention in patients with a prior SB and no other indication for testing, compared to patients with other indications for APT. From 1/87 through 4/91 there were 355 patients tested that had a prior SB with no additional risk factors. Of these, 299 delivered prior to 41 weeks and 56 had gestations that exceeded 41 weeks. Onset of testing ranged from 28 wks to 42 wks, with a median of 35 wks. The earliest intervention occurred at 35 weeks, with the majority occurring after 37 completed weeks(39/42). The comparison group was 979 consecutive patients being tested for postdates(PD), diabetes(DM), IUGR, HTN or decreased fetal movement(DFM). Patients were tested once or twice weekly with NST & AFI or biophysical profile. Significant variable decelerations (FHR fall >30 bpm & up to 15 sec or any fall lasting >1 min), late decelerations, or AFI < 5cm led to further evaluation on L&D for delivery. Significantly less intervention occurred for abnormal testing in patients with prior SB delivering prior to 41 weeks 24/299 (8%) than PD 118/497 (24%) $p < 0.00001$ or DM 46/232 (20%) $p < 0.0001$. Intervention rate was not different than DFM 6/121 (5%) $p < 0.37$, HNT 4/19 (21%) $p < 0.24$, or IUGR 15/110 (13.6%) $p < 0.13$. The intervention rate in patients with prior SB & PD 18/56 (32%) was not different than those with only PD 118/497 (24%) $p < 0.2$. This data shows that patients with a prior SB as the only indication for APT have significantly less intervention than patients with DM or postdates, and an intervention rate similar to patients tested for IUGR, HTN and decreased fetal movement. Patients with a prior SB whose gestation exceeds 41 weeks have a similar intervention rate as those patients whose only risk factor is postdates.

502 **MEAN AMNIOTIC FLUID INDEX BY GESTATIONAL AGE IN DIABETIC AND NON-DIABETIC PREGNANCY.** Montgomery DM*, Perlow JH*, Morgan MA, Nageotte MP, Garite TJ. Long Beach Memorial Womens Hospital. Long Beach, California University of California, Irvine Med. Center, Orange, California

The amniotic fluid index (AFI) has become an integral part of antenatal fetal assessment. However, presently there are no published data regarding the mean AFI at varying gestational ages in the pregnant diabetic. The objective of the study was to construct an AFI nomogram for the diabetic and non-diabetic populations at our institution. From 1/1/88-12/31/90, 249 diabetics (classes A-R) had a total of 1,506 AFI measurements at varying gestational ages. These values were compared with a control group consisting of 1,518 patients who had a total of 6,494 AFI measurements at corresponding gestational ages.



Statistically significant increases in mean AFI were demonstrated at 32, 33, 36 and 37 weeks gestation between the diabetic and non-diabetic patients ($p < .05$), the maximum difference observed was 1.2 cm. These data provide the basis for future studies to validate the use of the AFI in the diabetic pregnancy.

503 HOW FREQUENTLY SHOULD AFI'S BE REPEATED D. Lagrew, R. Pircon, M. Nageotte, R.K. Freeman, W. Dorchester*, Dept Ob/Gyn, Long Beach Memorial Medical Center, Long Beach, CA, Univ. of California, Irvine, Orange, CA.

The amniotic fluid index (AFI) has become a widely utilized technique for assessing fetal well-being. Intervals for measurement and cutoffs for intervention have been established by clinical experience. We analyzed AFI's on patients undergoing serial surveillance in order to evaluate once versus twice weekly measurements with respect to the chance of developing oligohydramnios (AFI < 5 cm) at each interval according to the initial AFI measurement. There were 11,759 AFI's of which 7,393 had a subsequent measurement within 4 days and 8,094 in 7 days. Initial AFI's were compared with the subsequent lowest value at 4 and 7 day intervals. If the initial AFI was < 5.0 cm, 59.4% had persistence of this low level 4 days later. If the initial AFI was 5 to 8 cm. only 5.4% had subsequent measurements of < 5.0 cm 4 days later. AFI's < 5.0 cm 4 days after an initial AFI of 8 to 15 cm occurred only 0.6% of the time and only 0.4% if the initial measurement was 15 to 25 cm. A similar trend was noted at 7 day intervals. These results suggest. the following: 1) an increased chance of developing oligohydramnios is present if an initial AFI is 8 cm or less. 2) Values above 8 cm are associated with a very low risk of oligohydramnios occurring in < 7 days and need not be repeated at < 7 day intervals.

504 **ANTEPARTUM SURVEILLANCE SHOULD BEGIN PRIOR TO 42 WEEKS (294 DAYS FROM THE LAST MENSTRUAL PERIOD) IN PROLONGED PREGNANCY.** M. Druzin, W. Wagner[X], S. Inglis[X]. NY Hospital-Cornell Med Center, NY,NY

The post term pregnancy (PTP) is defined as ≥ 42 weeks from the last menstrual period (294 days). There are numerous reports of fetal compromise after 40 weeks (280 days) and prior to 294 days in prolonged pregnancy (PP). Antepartum fetal monitoring was begun at 41 weeks (287 days) on 1080 patients. Gestational age was confirmed by sonography. Nonstress test (NST) was used as the primary test, and contraction stress test and biophysical profile as back-up for abnormal testing. Pregnancy outcome was evaluated on deliveries within seven days of the last NST.

	PP 41-41.6 wk n = 868	PTP ≥ 42 wk n = 212
C/S Abnl. Fetal Heart Rate	255/868 (30%)	23/80 (29%)
Meconium	119/299 (40%)	58/212 (27%)
Small for Gestational Age Fetus	19/868 (2%)	4/212 (2%)
APGAR 5 ' < 7	1/868 (0.1%)	1/212 (0.5%)
Abnormal Fetus	0/868	0/212
Perinatal Mortality	3/868 (0.3 %)	0/212
	All P NS	

Results: There were 868 patients with PP, 212 patients with PTP. There were no differences in labor and delivery course or neonatal outcome between the two groups. **Summary**: 1) Intrapartum fetal compromise (abnormal fetal heart rate requiring emergency delivery and/or meconium passage) was no different in the two groups. 2) Antepartum fetal heart rate monitoring should begin prior to 42 weeks.

505 **OLIGOHYDRAMNIOS AS AN INDICATION FOR RECOMMENDING DELIVERY IN POSTDATES PREGNANCIES.** C. O'Reilly-Green[X], M. Divon, Albert Einstein College of Medicine, Bronx, NY.

Oligohydramnios is a marker of adverse fetal outcome in postdates patients. Purpose: To evaluate the consequences of recommending delivery of postdates patients with oligohydramnios (i.e., amniotic fluid index (AFI) less than 5cm). AFI was prospectively obtained in 635 patients over 41 weeks' gestation. Indications for recommending delivery included a favorable cervix, poor fetal testing and oligohydramnios. 56 patients (9%) had a sonographic diagnosis of oligohydramnios. The control group was composed of 102 patients matched for gestational age without oligohydramnios.

	Low AFI	Normal AFI	p value
Intervention	48/56	46/102	.000002
Oligohydramnios (on R.O.M.)	27/51	10/100	.000001
Thick meconium	8/51	8/100	NS
Cesarean section	23/56	31/102	NS
For dysfunctional labor	7/56	20/102	NS
For uncertain fetal status	16/56	11/102	.05
Low Apgar, low pH or NICU admission	13/51	15/100	NS

In conclusion, these preliminary results suggest that recommending delivery of postdates patients with oligohydramnios does not result in a significant increase in the incidence of cesarean section yet provides fetal outcome comparable with that measured in the controls.

506 AMNIOINFUSION DOES NOT ALTER THE LENGTH OF LABOR.
C. J. Macri, D. B. Schrimmer, J. S. Greenspoon, T.H. Strong, R.H. Paul, University of Southern California, Los Angeles, CA

Amnioinfusion is effective in improving the pregnancy outcome in pregnancies complicated by repetitive variable or prolonged decelerations of the FHR, preterm premature rupture of the membranes, meconium, and oligohydramnios. The effect of amnioinfusion on the length of labor has not been described. We prospectively, randomly compared the length of labor in 437 patients. Patients who received amnioinfusion were compared to those without amnioinfusion. Patients were included in the study for one of two indications: 1) prophylactic amnioinfusion for oligohydramnios (AFI< 5 cm) (178 treatment, 89 control); 2) thick meconium and oligohydramnios (85 treatment, 85 control). There was no significant difference between the lengths of labor in the subgroups determined by method of delivery. Patients receiving amnioinfusion were significantly more likely to deliver vaginally (p<0.001).

Variable	Treatment AI	Control No AI	P
No. Patients	263	174	
No. (%) with:			
vaginal delivery	189 (71.8%)	96 (55.2%)	.001
forcep delivery	17 (6.5%)	18 (10.3%)	NS
vacuum delivery	17 (6.5%)	11 (6.3%)	NS
Cesarean delivery	40 (15.2%)	49 (28%)	.05
Length of Labor(hrs)mean(SD)			
vaginal delivery	9.3 (6.3)	10.6 (6.9)	NS
forcep delivery	8.4 (3.2)	9.1 (3.4)	NS
vacuum delivery	11.2 (5.8)	9.3 (3.5)	NS
Cesarean delivery	11.1 (6.3)	13.0 (7.5)	NS

507 A COMPARISON OF COMPLIANCE AND ACCEPTABILITY OF TWO FETAL MOVEMENT COUNTING METHODS. MC Freda[X], MS Mikhail[X], E Pollizotto[X], E Mazloom[X], IR Merkatz. Dept. OB/GYN, Albert Einstein College of Medicine, Bronx, NY.

Fetal activity is a sign of fetal well-being.Fetal movement counting is a simple, non-invasive, cost effective method of fetal surveillance used to estimate fetal well-being which has recently been shown to enhance maternal-fetal attachment.Two methods of fetal movement counting are most often mentioned in the literature, with each claiming unique advantages over the other.Studies have not been done to determine which method works best or is most acceptable to women.To answer these important questions, a randomized study was done.All women had singleton, uncomplicated pregnancies between 28-32 weeks.Group A (n=63) used the Sadovsky method (counting 4 fetal movements 3 times a day).Group B (n=62) used the Cardiff method (counting the first 10 movements each morning).All women agreed to count for 1 month, then completed a 9 question acceptability survey.An expert panel reviewed each graph for quality and compliance, assigning a score of 1 (excellent) through 5 (poor). Acceptability of the method was assigned a score based on answers to the survey.Acceptability of the method was significantly associated with the quality of the graph (p<.025) regardless of the method used.Maternal age, education, race, marital status or employment did not mediate acceptability. There were no statistically significant differences in quality scores between the two methods. Results suggest that inner city patients can select either method of fetal movement counting and achieve the same level of compliance and quality of reporting.

508 **EFFECT OF ALTITUDE ON THE AMNIOTIC FLUID INDEX.** M.K. Yancey,[X] D.S. Richards, Department of Obstetrics and Gynecology, University of Florida, Gainesville, FL.

Altitude is known to effect fetal size and other aspects of maternal/fetal physiology. The effect of altitude on amniotic fluid volume has not previously been studied. The amniotic fluid index (AFI) is a reproducible means of assessing amniotic fluid volume. We studied the effect of altitude on the AFI by comparing similar populations of patients at low altitude (100 ft.) and high altitude (6000 ft.). A standard technique of measurement of the greatest vertical pockets of fluid in each quadrant with summation of values was utilized. Inclusion criteria included an uncomplicated singleton pregnancy between 20-42 weeks gestation with adequate dating criteria. Women were excluded from the study if any condition known to alter amniotic fluid volume was present. Patients were grouped by gestational age at two week intervals. Statistical analysis was performed with ANOVA. The population at high altitude (N = 364) had a significantly ($P < 0.0001$, $R^2 = 0.21$) increased AFI throughout gestation compared to the low altitude group (N= 514). The mean difference in the AFI between the two groups was 2.6 cm (range 1.1 - 3.6 cm for the various interval groups). We conclude that the amniotic fluid volume in normal pregnancies appears to be increased at high altitudes. The mechanism for this increase is unknown.

509 **PREGNANCY OUTCOME BY THE DEGREE OF MATERNAL SERUM ALPHA FETOPROTEIN ELEVATION.** W. Cusick[X], J. Rodis, A. Vintzileos, M. Albini[X], M. McMahon[X], W. Campbell, Univ. of CT Health Center, Farmington, CT

This retrospective study of pregnancies with unexplained second trimester elevated MSAFP sought to: 1) determine if a correlation exists between the degree of MSAFP elevation and adverse pregnancy outcome (intrauterine growth retardation, preterm births and pregnancy loss); 2) examine the timing of losses in pregnancies with bad outcome; and 3) develop a protocol for antepartum fetal surveillance in an effort to prevent these adverse outcomes. Well dated, singleton pregnancies with a single elevated MSAFP ($\geq$2.0 MOM) were eligible if targeted ultrasound elevation ($\leq$22 weeks) agreed with LMP dating and revealed no fetoplacental anomaly. A total of 371 patients were enrolled; delivery data was available on 337 infants. Stratified by MSAFP elevations of 2.0-2.49, 2.50-2.99, and $\geq$3.0 MOM, the rate of adverse outcomes were: 1) SGA (<10%ile) infants: 14/189 (7.4%), 10/91 (11.0%), 12/54 (22.2%); 2) preterm delivery (<37 weeks): 28/192 (14.6%), 15/91 (16.5%), 11/54 (20.4%); and 3) pregnancy losses (neonatal and intrauterine fetal deaths): 5/190 (2.0%), 3/89 (3.4%), 3/51 (5.9%), respectively. Seven early losses (3 fetal and 4 neonatal) occurred prior to 28 weeks. Of these seven, six fetuses exhibited IUGR by 23-26 weeks gestation and 5 of these 6 were associated with greater degrees of MSAFP elevation ($\geq$2.5 MOM). Four losses (2 fetal and 2 neonatal) occurred in the third trimester, including one neonatal death at 28 weeks with dextrocardia. Of these 4, the 3 structurally normal infants exhibited normal growth and were lost after 34 weeks gestation; all 3 of these pregnancies exhibited MSAFP elevations $\leq$2.5 MOM. This data suggest that any proposed program to improve pregnancy outcome in patients with unexplained MSAFP elevation must include: 1) repeat ultrasound evaluation at 24 weeks to rule out early IUGR in cases of MOM $\geq$2.5; 2) efforts aimed at preventing preterm delivery; and 3) fetal biophysical monitoring, even in normally growing fetuses, instituted at 32 weeks to detect fetuses at risk for intrauterine fetal death.

510 THE ROLE OF ANTENATAL TESTING IN THE CLINICAL MANAGEMENT AND OUTCOME OF FETAL ARRHYTHMIAS. A. Khoury,[X] M. Moretti, N. Meyer,[X] A. Nova,[X] T. DiSessa,[X] B. Sibai, P. Mace.[X] University of Tennessee, Memphis.

The purpose of this study is to report the clinical management and neonatal outcome in 18 fetuses with perinatally diagnosed cardiac arrhythmia. Two dimensional, guided M-mode echocardiography and pulsed doppler ultrasound studies were performed with commercially available scanners. The fetuses presented with various arrhythmias; PAC N=12, PVC N=1, A.Fib (2:1 block) N=2, A.Flutter (2:1 block) N=1, SVT N=2. All fetuses were devoid of structural heart disease. Antenatally the fetuses with PAC, and PVC were followed weekly with biophysical testing, the remainder were followed twice weekly. Results: mean gestational age at time of diagnosis was 30.1 week (range, 24-38), the mean number of antenatal tests used were 4.1 (range, 1-13), none of the fetuses had evidence of hydrops on examination. Four fetuses required treatment with digoxin; one fetus with SVT converted to normal rhythm and was delivered vaginally. Three were delivered by cesarean section for fetal arrhythmia. Two of these fetuses converted to normal rhythm spontaneously within 24 hours of birth. The third fetus (A. Fib) required transesophageal pacing in the first day of life. The mean gestational age at time of delivery 38.8 weeks (range, 37-40). Mean birth weights 3125 gm (range 2450-3685), all Apgars at 5 minutes were normal, mean 8.92 (range, 8-9), none of the neonates required prolonged hospitalization, mean 2.1 days (range 1-5). There were no neonatal deaths. Conclusion: Intensive and close antepartum testing is warranted in cases of SVT A.Fib & A.Flut., while routine follow-up for PAC, and PVC is adequate since the incidence of congenital heart lesions is low in this group of PA. Moreover all fetuses with PAC and PVC revert to normal rhythm in the neonatal period spontaneously and without medical treatment.

511 FETAL CATECHOLAMINE RESPONSES TO VIBRACOUSTIC STIMULATION K. Murphy,[1x] K. Hanretty,[1x] G. Inglis,[2x] A. Cameron,[1]
The Queen Mother's Hospital [1] *& MRC Blood Pressure Unit* [2], *University of Glasgow, SCOTLAND.*

The purpose of this study was to determine the role of the adrenal medulla in mediating fetal response to vibroacoustic stimulation. Forty-eight mothers undergoing elective Caesarean section at term under regional anaesthesia were randomly allocated either to a group who received fetal vibroacoustic stimulation 1-2 minutes prior to delivery of the infant, or to a group of controls. A 70 dB vibroacoustic stimulus lasting 3 seconds was applied to the maternal abdominal wall overlying the fetal head 110 (SD 49) seconds before delivery in 25 infants, and the levels of noradrenaline (NA), adrenaline (A) and renin (R) in both umbilical arterial and venous blood of these fetuses were compared with those from 23 controls. The two groups were comparable in all respects except for the stimulus. The median value for NA in the umbilical artery in the stimulated group (6.6 nmol/l) was not significantly different from that (8.4 nmol/l) in the control group (Mann-Whitney test; n=42, p> 0.05). Similarly, no differences were found for A, or R in either the umbilical artery or vein between the two groups, or for NA in the umbilical vein. Subjects who had hypotension (systolic BP <90 mmHg) induced by their regional block, particularly spinal anaesthesia, had significantly higher levels of NA in the umbilical artery at delivery compared with those who remained normotensive (median value 10.5 vs 5.5 nmol/l, $p < 0.05$). In addition there was a weak, but statistically significant correlation between umbilical artery NA levels, and the dose of ephedrine used to treat maternal hypotension (Spearman Rank correlation ; $r = 0.367$, n=42, $p < 0.05$). We conlcude that vibroacoustic stimulation does not induce fetal behavioural changes via a surge of catecholamines from the adrenal medulla, though fetal NA levels may be influenced by maternal blood pressure and ephedrine.

512 **SURVEY ON THE MANAGEMENT OF POST DATE PREGNANCY**

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Management strategies for prolonged pregnancies remain controversial. We conducted a survey of SPO members to determine the current strategies employed in the management of these patients. Material and methods: In November 1990 a questionnaire was mailed to 1,000 members of SPO. Six hundred and seventy seven (68%) completed questionnaires were returned. Results: The results in Table I reflect managements employed according to gestational age, with certain dates, and the condition of the cervix as judged by the Bishop Score (BS). Table II reflects the type of antepartum testing used. Table III reflects agents used for cervical ripening. Table IV reflects modes of induction. When dates were uncertain, 614 (91%) of the responders would intervene only if antepartum testing was abnormal. Finally, only 313 (46%) of responders had a written protocol for management of these patients.

Table I

	287 days *BS > 5	287 days BS ≤ 5	294 days BS > 5	294 days BS ≤ 5
Induction	65%	6%	97%	56%
Antepartum testing	27%	83%	1%	38%
No action	7%	10%	1%	4%
No response	1%	1%	1%	2%

Table II	Table III	Table IV
NST - 71%	PGE2 - 58%	Anmiotomy - 14%
CST - 4%	Oxytocin - 7%	Oxytocin - 34%
BPP - 21%	Laminaria - 7%	PGE2 gel - 1%
	Combination - 24%	Combination - 50%

Conclusion:
The majority of SPO members will induce labor at 287 days if the cervix is favorable and dates are certain. If the cervix is unfavorable, however, the responders initiate antepartum testing at 287 days and induce labor at 294 days. On the other hand when dates are uncertain, intervention is recommended only if antepartum testing is abnormal.

*BS = Bishop Score

513 ALPHA-FETOPROTEIN LEVELS FOLLOWING MATERNAL TRAUMA

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Maternal serum alpha-fetoprotein (AFP) has been proposed to assess fetal risk following maternal trauma. Serum specimens were obained at evaluation for trauma beyond 20 weeks gestation. Pregnancy outcome was evaluated. AFP levels were measured using an enzyme immunoassay. Complete data was available for 107 women. Mean and median AFP levels were 213.1 and 165.2 ng/ml, repectively. There was no linear trend for AFP level with gestational age (r=0.01; p=0.89). Fourteen women had AFP levels > or = 300 ng/ml. Outcome measures were similar for these women compared with the remainder:

AFP	<300	>300	Total
n	93	14	107
Delivered <37 weeks	17 (0.18)	3 (0.21)	20
Birth weight <2500 g	13 (0.14)	1 (0.07)	14
Apgar(5) < 7	2 (0.02)	1 (0.07)	3
Interval <7 days	14 (0.15)	2 (0.14)	16

(Interval = number of days from the trauma episode until delivery) The AFP level was 144.9 ng/ml in the one perinatal death due to abruption after trauma. Three women had levels > 800 ng/ml. Their complications were: labor at 36 weeks; marginal abruption and delivery at 35-36 weeks; and, induced delivery at term for positive fetal cell test. Very high AFP levels (> 800 ng/ml) may predict complications after trauma.

514 THE RELATIONSHIP BETWEEN THE RATE OF FETAL URINE PRODUCTION AND THE AMNIOTIC FLUID INDEX IN PREGNANCIES ≥ 38 WEEKS.

LJ Groome, FL Gaudier, JC Hauth, CL Neely[x], SP Cliver[x], J Owen. University of Alabama School of Medicine, Birmingham, Alabama.

It is often assumed that a normal amniotic fluid volume (AFV) is evidence of adequate fetal renal function, the implication being that AFV is a reliable measure of fetal urine production. Purpose: to determine the relationship between AFV and the hourly fetal urine production rate (HFUPR) in 134 normal pregnancies ≥ 38 weeks. The amniotic fluid index (AFI) was used as a clinical estimate of AFV; the HFUPR was determined by measuring the fetal bladder volume every 3-5 min for a period of 20-30 min. When controlled for gestational age, we found no correlation between the HFUPR and the AFI. However, a statistically significant ($p = 0.006$) relationship was found between an HFUPR below the tenth percentile (in this study, an HFUPR ≤ 30 ml/hr) and a clinical diagnosis of oligohydramnios. For the purpose of identifying fetuses with an HFUPR ≤ 30 ml/hr, an AFI ≤ 8 cm had a sensitivity of 71%, a specificity of 67%, a positive predictive value of 24%, and a negative predictive value of 94%. Conclusion: an AFI > 8 cm is a reasonable criteria by which to exclude the fetus with an abnormally low rate of urine production.

515 COMPUTERIZED VS. VISUAL ANALYSIS OF FETAL HEART RATE: A REDUCTION IN TESTING TIME.

Karin A. Blumofe[x], BA, Paula M. Broussard[x], RN, BS, Catherine A. Walla[x], MA, MN, Lawrence D. Platt, MD. Department of OB/GYN, Cedars-Sinai Medical Center, Los Angeles, CA.

A study was performed to determine if computerized analysis of fetal heart rate (FHR) tracings as opposed to visual interpretation can decrease the mean testing time in antepartum fetal surveillance. Eighty-one high-risk gravidas underwent 152 nonstress tests utilizing the Oxford Sonicaid System 8000. A FHR record was determined to be normal by this system according to the following criteria: FHR variation > 30 msec; the presence of ≥ 3 accelerations (>10 bpm above the baseline and a duration > 15 secs) or 1 maternally perceived fetal movement; and the absence of large decelerations. Criteria for reactivity by visual analysis included the identification of ≥ 2 accelerations ≥ 15 bpm above the baseline with a duration of ≥ 15 secs within a 10-minute period. Patient management was based on the visual interpretation of the FHR tracings. Results:

	Normal Test Results N (%)	Length of Test
Computer Analysis	145 (95.4)	16 ± 9 mins
Visual Analysis	138 (90.8)	35 ± 17 mins
p	0.50	< 0.0001

Computerized analysis of the FHR tracings provides interpretations comparable to those given by visual analysis while significantly decreasing the mean testing time.

516 A FOUR YEAR FOLLOW-UP OF HEARING AND NEURO-DEVELOPMENT IN CHILDREN EXPOSED IN UTERO TO VIBROACOUSTIC STIMULATION

By Westgren M, Nyman M, Barr M, Dept Obstet Gynecol Karolinska Institutet Huddinge University Hospital, Sweden

Several investigators have expressed concerns about exposing the fetus to VA stimulation we found it therefore essential to study the long-term effect.

A total number of 525 children were included in the present study. All children had been followed according to the routine Swedish health care program. At 4 years of age an extensive examination is performed including an auditory test (250-8,000 Hz).
Results: In this material two children had hearing defects (otosalpingitis, hereditary). Eight children were disabled, but no handicap could be related to the VA stimulation.
Discussion: This study does not provide any evidence that VA stimulation should be associated with an increased risk of neuro-developmental disorders nor impaired hearing.

517 **POSITION OF THE VIBROACOUSTIC STIMULATOR DOES NOT AFFECT FETAL RESPONSE.** **DP Eller, RB Newman, L Johnson[X]. Medical University of SC, Charleston, SC.**

The fetal vibroacoustic stimulation test (VAST) has become an established adjunct to the nonstress test (NST) for assessment of fetal well-being . However, positioning of the stimulator over the fetal vertex was empirically selected with little consideration for alternatives.The optimal placement of the vibroacoustic stimulator has not been established. Gerhardt measured extremely intense sound pressures (135 dbs) when an electronic artificial larynx (EAL) was applied directly over the fetal ear in pregnant ewes (AJOG1988; 159: 228-32). Sound pressures decreased as the distance from the EAL increased. The following study prospectively evaluated the fetal response to stimulation randomly applied over the fetal vertex or breech. The parameters evaluated include fetal heart rate (FHR) reactivity, fetal movement (FM) and fetal startle. Between December, 1990 and May, 1991, 205 patients with a nonreactive NST after ten minutes were prospectively randomized to receive VAST (Corometrics Model 145) over the fetal vertex (n=110) and over the fetal breech (n=95). Both groups were similar with respect to maternal age, gestational age, and indication for NST. A subset of 49 patients (24 breech and 25 vertex) was evaluated for a change in FM with VAST as recorded by a doppler fetal activity monitor (Actograph MT-320, Toitu Corp.). The fetal startle response (characterized by sudden gross body movements and flexion-extension of all extremities lasting 5-10 seconds after VAST) was observed ultrasonographically in 20 additional patients (10 breech and 10 vertex). Chi square analysis revealed virtually identical FHR responses in both groups. The increase in the number of FM after VAST compared to the pre-stimulation baseline was not different between groups. The fetal startle response was uniformly observed in both groups. VAST over the fetal breech elicits an identical short term fetal response compared to stimulation over the fetal vertex with a potentially less intense sound exposure. Until more is known about the long term effects of VAST on fetal hearing, stimulation over the breech may be preferable.

518 **FETAL HEART RATE ACCELERATIONS, FETAL MOVEMENT AND FETAL BEHAVIOR PATTERNS IN TWIN GESTATIONS.** **M.W. Gallagher,[X] T.R.B Johnson, Dept. Gyn/Ob, The Johns Hopkins Univ. Sch. of Med., Balto., MD. 21205**

Previous studies have shown that twins, when monitored simultaneously, show a remarkably high incidence of synchronous fetal heart rate accelerations (58%), prompting inquiry into the nature of the intrauterine interaction of twins. The present study is a retrospective examination of 20 fetal monitor strips from five sets of twins with simultaneous fetal heart rate and fetal activity recorded using a doppler fetal movement detector (Toitu MT-320-Fetal Actocardiograph). The strips were analyzed for coincidence of fetal heart rate accelerations and/or fetal movement episodes. Forty-eight percent of fetal heart rate accelerations were found to be simultaneous. Fifty-seven percent of movement epochs were considered simultaneous. The strips were then analyzed using concepts of fetal behavioral patterns based on descriptions of behavioral states by Prechtl and Nijhuis. We found that twins exhibited coincident behavioral patterns (basically sleep/awake state) 88% of the time. Twin-twin interactions are more consistently related than suspected when observation is limited to heart rate alone and the coincidence of and role of fetal behavioral states must be considered when twin interactions and behavior are studied.

519 AMNIOTIC FLUID VOLUME ESTIMATION IN THE POSTDATES PREGNANCY: A COMPARISON OF TECHNIQUES.

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A number of techniques for amniotic fluid volume (AFV) estimation have been proposed, including the largest vertical pocket (LVP) and amniotic fluid index (AFI). Oligohydramnios has been variously defined as LVP < 1 or 2 cm, or AFI <5 cm or 2.5th percentile. Purpose: To determine the AFV technique that was most predictive of abnormal perinatal outcome in 137 postdates pregnancies. Gestational dating was based on an early ultrasound (≤26wks) which either confirmed or established the EDD, or a late ultrasound that was consistent with or greater than the menstrual age. With the ultrasound transducer in a longitudinal plane, the largest amniotic fluid pocket in each quadrant was measured in both vertical and transverse dimensions. The last AFV prior to delivery was correlated with perinatal outcome. Outcome was considered abnormal for: 1) operative delivery for non-reassuring FHR tracing, 2) meconium below the cords, 3) 5 minute Apgar <7, 4) umbilical artery pH <7.10 or venous pH <7.15, 5) admission to NICU, or 6) BW < 10%. A receiver operating characteristic (ROC) curve was employed to determine the optimal LVP and AFI. Results: The mean LVP was significantly lower in the abnormal outcome group compared to the normal group (3.4 ± 2.0 vs 4.2 ± 1.4 cm, p = .04), whereas no significant difference was noted using the AFI (8.7 ± 5.5 vs 9.7 ± 4.3 cm, p = .24).

AFV Test	Normal Outcome (N=103)	Abnormal Outcome (N=34)	p
LVP <1 cm	0 (0%)	3 (8.8%)	.01
LVP <2 cm	3 (2.9%)	8 (23.5%)	.0006
ROC LVP <2.5 cm	8 (7.8%)	12 (35.3%)	.0003
AFI <5 cm	12 (11.7%)	8 (23.5%)	.10
AFI <2.5%	21 (20.4%)	11 (32.4%)	.15

We conclude from our study of postdates pregnancies that: 1) an LVP <2.5 cm is the most useful AFV indicator of subsequent abnormal perinatal outcome, with a sensitivity of 35.3% and a specificity of 92.2%, and 2) the AFI has little diagnostic value in this population.

520 ANGIOTENSIN TESTING PREDICTS FETUSES BENEFITTING FROM LOW DOSE ASPIRIN. BJ Trudinger, C-M Cook[X], Dept. Ob/Gyn, Univ. Sydney, Westmead Hospital, Westmead, Australia.

Low dose aspirin improves fetal growth when used to treat placental insufficiency identified by an abnormal umbilical Doppler study, and a positive angiotensin infusion sensitivity test (AIST) identifies fetuses with an abnormal study at greatest risk of subsequent morbidity, even in the absence of maternal hypertension. We therefore investigated the effect of aspirin therapy in a group of mothers without hypertension identified by a high umbilical S/D and a positive AIST response (a group with a poor fetal prognosis). From 604 high fetal risk pregnancies 40 were found to have a high umbilical S/D of whom 23 exhibited a positive response to AIST. Gestational age at enrolment ranged from 26 to 35 weeks. This group was treated with aspirin 100mg/day. The AIST was repeated after at least 7 (range 7-23) days. In all 7 fetuses with absent diastolic flow the test remained positive. In the remaining 16 a negative repeat study (n=6) was associated with a decrease towards normal in umbilical S/D. The mean centile birthweight was less (6 to 26, $p<0.005$) and mean gestation at delivery earlier (35.1 to 38.6 wks, $p<0.001$) in the positive compared to negative second AIST result groups. The group with an intial negative AIST were not treated with aspirin and their outcome was comparable to the negative restudy group. We conclude that a positive AIST predicts the true positive high umbilical S/D fetuses with vascular disease in the fetal placenta and this response remains positive if aspirin fails to arrest the placental obliterative vascular pathology.

521 **VIBRATORY ACOUSTIC STIMULATION STIMULATES HUMAN FETAL VOIDING.** E. Z. Zimmer[X], C. R. Chao[X], G. P. Guy[X], F. Marks, W. P. Fifer[X], Dept. of Ob/Gyn, Columbia University, New York, NY

Although vibroacoustic stimulation (VAST) is a popular adjunct to fetal heart rate testing, its effects on many fetal organ systems are unknown. We hypothesized that VAST might stimulate fetal micturition. Fetal bladder volume was determined by ultrasound in 20 healthy fetuses at 38-41 weeks gestation. Measurements were taken 5 minutes prior to and immediately preceding a 3-second VAST and at 1 and 5 minutes following the VAST. All stimuli were performed during the filling phase of the bladder cycle; i.e., the volume immediately prior to VAST was greater than at 5 minutes prior to VAST.

Bladder Volume (ml)	Mean	SEM	Group*
5 minutes prior	17.7	2.1	a
Immediately prior	22.7	2.4	b
1 minute after	14.2	2.2	a
5 minutes after	13.3	1.9	a

*$p=0.001$, repeated measures ANOVA. Groups with same letter are not different by Neuman-Keuls post-hoc analysis at $p<0.05$; groups with different letters are significantly different at that level

Mean bladder volume was significantly decreased 1 minute and 5 minutes following the VAST compared to the volume immediately prior to the stimulus. The volume decrease was observed in 19 of the 20 cases at 1 minute following VAST. We speculate that fetal micturition following VAST may be part of a fearful reaction to the stimulus.

522 ELEVATED MSAFP AND RISK ASSESSMENT FOR PREGNANCY OUTCOME.
W Moroder*, RR Viscarello, S Yarkoni, D Brioschi*, and JC Hobbins, Dept. of OB/GYN, Yale University Sch. of Medicine, New Haven, CT.

Although elevated levels of MSAFP in pregnancy are associated with congenital anomalies, pre-eclampsia, low birth weight, and fetal death, it is difficult to assign accurate risks for these outcomes. The purpose of this study was to examine the relationship between elevated MSAFP levels and the risk of adverse pregnancy outcome. During a 2 year period, 439 patients with MSAFP levels >2.0 MOM were referred for targeted ultrasound examination. Sonographic findings and pregnancy outcome data were retrospectively reviewed in 355 patients (81%). Fetal or placental anomalies were noted in 141 pregnancies (39.7%), including 33 structural defects and 7 chromosomal aberrations. Mean MSAFP levels were significantly higher in patients with fetal anomalies (4.9 MOM vs. 2.6 MOM; $p< 0.01$). Placental abnormalities were detected in 14% of patients and were associated with a mean MSAFP value of 2.97 (range: 2.0 to 11.4). While 3.4% of pregnancies had oligohydramnios and 1.4% had polyhydramnios (mean MSAFP = 6.6 MOM), only the former was associated with a lower mean birthweight (2431g vs. 3181g; $p< 0.01$). Pregnancy complications included 6 cases of intrauterine fetal demise, 38 premature deliveries, and 34 patients with PIH (10%). Patients with pre-eclampsia had a mean MSAFP value of 2.6 MOM, which correlated with a significantly lower birthweight ($p< 0.005$). Of note, hemangiomas were reported in 23 neonates (7%) who were born to mothers with otherwise uncomplicated pregnancies, which suggests that these birthmarks have been a previously unrecognized cause of elevated MSAFP values. Our data confirm previous observations that an unexplained, elevated MSAFP level is a marker for high risk pregnancies. In addition, the increased risk for fetal and/or placental anomalies suggests that a targeted ultrasound examination is warranted in all patients with elevated levels of MSAFP.

523 **RELATION OF MILD IDIOPATHIC POLYHYDRAMNIOS TO PERINATAL OUTCOME.** **C.Smith, R.Plambeck[X], W. Rayburn, K. Albaugh[X], Dept of OB/GYN, Univ of Nebraska College of Medicine, Omaha, NE.**

The relation between clinically obvious polyhydramnios and poor perinatal outcome is well established. Much less is known about mild unexplained polyhydramnios, which usually is initially suggested by sonographic examination late in gestation. The purpose of the present investigation was to relate mild idiopathic polyhydramnios to perinatal outcome. Mild polyhydramnios was defined sonographically as an amniotic fluid index of 25-39 during fetal biophysical testing. All cases involved single gestations not complicated by conditions known to predispose to polyhydramnios. Mild polyhydramnios was diagnosed in 97 (8.2%) of 1177 patients undergoing fetal testing between 26 and 42 gestational weeks. Findings of premature delivery, intrapartum complications, and neonatal depression were no more frequent in pregnancies complicated by mild, unexplained polyhydramnios than in a comparable control group of patients but with a normal fluid volume. The incidence of birth weights greater than 4,000 grams was significantly higher in the mild polyhydramnios group than in the control group (18.6% vs 8.6%; $p<0.05$). We conclude that mild idiopathic polyhydramnios based on sonographic examination in late gestation is not associated with an increased risk of adverse perinatal outcomes, except for a higher incidence of large-for-gestational-age fetuses.

524 ABSENT UMBILICAL ARTERY END DIASTOLIC VELOCITY(AEDV) AND REVERSE FLOW - CLINICAL OUTCOME OF 60 CASES. JG Bell[X], A Ludomirsky[X], J Bottalico[X], S Weiner, Pennsylvania Hospital, Philadelphia, PA

We studied our experience of 60 cases with a diagnosis of AEDV(n=50) or reverse flow(n=10). Mean time from onset of AEDV to delivery was 19.1 days(range 0-107). Gestation at diagnosis ranged from 18 to 34 weeks. The mean time from diagnosis of AEDV to reverse flow was 16.3 days. Management of AEDV included hospitalization with bed rest, oxygen therapy, and intensive fetal monitoring. 8 of 9 fetuses with reverse flow at viable gestational ages were delivered on the day of diagnosis. Indications for delivery with AEDV included abnormal NST/CST in 56%, maternal indications in 22%, and others in 12%. Karyotypes were available on 22 fetuses with 9% abnormal. Major structural anomalies were present in 4 others. Pathology revealed infarctions in 46%of placentas. Of 16 arterial cord pH values available, mean pH was 7.27.

	AEDV	Reverse Flow
IUGR by US(<10%)	26/50(52%)	8/10(80%)
Oligohydramnios	22/50(44%)	2/10(20%)
Avg.Birthwt.(gms)	1076(range 450-2400)	930(280-1879)
Neonatal Death	10/50(50%)	3/10(30%)
Survivors	33/50(66%)	6/10(60%)
# Days in Nursery	55(range 5-186)	65(44-126)
IVH	3/50(6%)	1/10(10%)

Conclusion:Similar perinatal and necnatal outcomes were found in both AEDV and reverse flow, and were not as poor as those previously reported in the literature.

525 **ROLE OF THE ABBOTT TDxFLM ASSAY IN ASSESSING FETAL LUNG MATURITY (FLM)** MM Schnoor[X], WNP Herbert, JF Chapman[X], Dept. Ob/Gyn and Path, UNC School of Medicine, Chapel Hill, NC.

The TDxFLM test (Abbott Laboratories, Abbott Park, IL 60064) is an automated test for FLM based on quantitative fluorescence polarization. In 102 patients, 22 of whom had infants with RDS, we assessed the TDxFLM test with respect to other tests (L/S, FSI, and Amniostat-PG [Irvine Scientific, Santa Ana, CA 92705]) and respiratory outcome. We also evaluated various strategies for FLM testing using sequential and parallel approaches. In comparing tests, the TDxFLM (referent value 30 mg/g) had the highest sensitivity (SE), specificity (SP), predictive values (FVm mature, PVi immature) and efficiency (EF). For sequential and combination testing, the FLM (if negative) followed by the FSI was comparable to other combinations in SE, SP, PV, EF, and cost.

Test(s)	SE(%)	SP(%)	PVm(%)	PVi(%)	EF(%)	Cost
Individual Tests & Referent Values for Maturity						
TDx (≥30mg/g)	100	82.5	100	61.1	86.3	$30
L/S (≥2.0)	86.4	72.5	95.1	46.3	75.5	$32
FSI (≥48)	86.4	77.5	95.4	51.4	79.4	$ 8
AMN-PG (≥wk+)	100	36.3	100	30.1	50.0	$28
Multiple Testing Approaches (sequential vs. combination)						
FLMi then FSI	86.4	95.0	96.2	82.6	93.1	$32
FSIi then L/S	77.3	87.5	93.3	63.0	85.5	$20
AMNi then FSI	86.4	86.3	95.8	63.3	86.3	$34
FSIm & L/Sm	95.5	62.5	98.0	41.2	69.6	$40

Conclusion: The Abbott TDxFLM Assay compares favorably with other FLM tests. Its simplicity, rapid analysis time, precision and clinical reliability warrant its strong consideration as a "first-line" test for FLM.

526 **FETAL HEART RATE PATTERNS AND SUBSEQUENT CEREBRAL PALSY: CONVERSION OF REACTIVE NST TO PATTERN OF CHRONIC DISTRESS.** Barry S. Schifrin, M.D., James R. Shields, M.D.[X] Department of Obstetrics and Gynecology. Tarzana Regional Medical Center, Tarzana, California

Some fetuses who develop cerebral palsy (CP) demonstrate during labor persistently absent variability, small variable decelerations with overshoot and absent asphyxia. We have interpreted this pattern as neurological injury antedating labor. To better understand its appearance, we analyzed the antepartum NST results from 44 CP patients with this pattern during labor. Decreased fetal movement, decreased amniotic fluid volume, meconium staining, and dysmaturity were common findings.

NST RESULT	NO.	%	DECELS	%
Reactive NST	34	77.3	8	23.5
Intermediate NST	6	13.7	1	16.7
Non-reactive NST	4	9.0	0	0.0
Total	44		9	

The conversion of the reactive NST with its normal fetal behavior pattern to a pattern of absent variability in the clinical setting of decreased fetal movement and/or oligohydramnios suggests that the neurologic injury in these cases is of relatively recent origin and that NST testing may be of value in timing the injury. The NST may also prompt timely intervention when decelerations, oligohydramnios, or intermediate results are present. The NST alone, however, does not anticipate the majority of the injuries in this unique group of patients. Evaluation of fetal movement and amniotic fluid volume may improve the prediction.

527 **DOES THE AMNIOTIC FLUID INDEX (A.F.I.) CHANGE OVER A SHORT TERM TIME INTERVAL?** D. Schwartz[1], Y. Daoud[1X], K. Schukter[2X], J. Freeman[2X], K. McGirr[2X], S. Campbell[2X].
[1] Sinai Hospital, Wayne State Univ, Detroit, MI., [2] Kings College Hospital, London, England.

The amniotic fluid index (A.F.I.) is commonly used to assess amniotic fluid volume, as the technique is relatively rapid and simple. This assessment is usually used in conjunction with other tests of fetal wellbeing that may extend over a variable time interval. The data on inter-observer and intra-observer variability has not stated the interval between measurements, although it is likely that they would have been repeated immediately. In this study an A.F.I. was repeated after a short time interval to assess for a significant difference, as this may be clinically relevant. In 91 patients, the same individual performed the test at the beginning and at the end of an ultrasound scan for either growth and development (43), or for doppler velocimetry (48). The gestational ages ranged from 20 to 41-3/7 weeks. The interval between the two measurements was 30 to 45 minutes. The two values were correlated and assessed for a significant difference using a paired t-test. For the two measurments, the respective results were as follows: mean 18.4 vs 18.7; standard deviation 4.2 vs 3.9; minimum value 10.1 vs 11.0 and maximum value 35.0 vs 30.0. There were no significant differences between the two measurements ($P>0.133$; df=90) and the correlation coefficient was 0.874 ($P<0.0001$). In 57 cases (63%), the difference was less than 10% and in 82 cases (90%), it was less than 20%. The current results validate the reproducibility of the AFI measurement over a 30-45 minute time interval, during which time most adjunctive tests associated with the A.F.I. are usually completed. Therefore, the timing of the A.F.I. in relation to the other tests is not critical and repeating the A.F.I. within 30-45 minutes of a preceding assessment is not necessary.

528 **PREDICTION OF LONG TERM NEUROLOGIC HANDICAP IN VERY LOW BIRTHWEIGHT NEWBORNS**

FL Gaudier,* RL Goldenberg, M Peralta,* KG Nelson,* M DuBard,* SE Johnson,* RA Steele,* TY Roth*
University of Alabama Hospitals, Birmingham, Alabama

This study was performed to determine if factors other than low BWT predict neurologic handicap. 310 infants with a BWT of 500-1000 gms who delivered between 1979-89, with a last evaluation at ≥1 year of age were studied. The factors studied included BWT, GA, Apgar score, and umbilical arterial cord gases including pH, HCO_3, pCO_2 and pO_2. Outcomes evaluated included mental retardation (MR) defined as an IQ <70 on the last IQ test performed, cerebral palsy (CP), and any major handicap (MH) including MR, CP, blindness, deafness and hydrocephalus. BWT was not associated with any of the outcomes studied, while the GA was inversely associated with CP (p=.05) and MH (p=.02). Both 1 and 5 minute Apgar scores were associated with MR (p=.02) and MH (p=.02). Both high and low pH values were associated with CP and (p=.003) and MH (p=.001). As an example, 5 of the 6 infants with a pH <7 had a MH (p<.001) compared to 20% with a pH >7 and <7.35. Of infants with a pH of >7.35, 50% had a MH (p<.0001). Levels of HCO_3 were inversely associated with all 3 outcomes (p≤.01). As an example, a HCO_3 of ≥26 was associated with 9% MH while a HCO_3 <14 was associated with 54% MH. Both high and low levels of O_2 and CO_2 were associated with the 3 poor outcomes. Regression analyses, controlling for GA, BWT, 1 minute Apgar score and type of anesthesia were performed to confirm the relationships between cord gases and outcome described above. As an example, the Odds Ratios (OR) for MH associated with a pH <7 was 4.1 and the OR for MH for a pH >7.35 was 3.9 compared to a pH of 7 to 7.35 . The OR for MH for a HCO_3 <13 compared to a HCO_3 of >23 was 6.4. The U-shaped relationship between both high and low CO_2 and O_2 and MH was confirmed. In summary, cord gas measurements are highly predictive of long term neurologic handicap in very low birthweight infants, but the relationships are more complex than originally anticipated.

529 OBSTETRIC PREDICTION OF THE SYMPTOMATIC GROWTH RETARDED NEONATE. H.M. Wolfe, M.P. Dombrowski, R.J. Sokol, Y.W. Brans[x], Dept. of Ob/Gyn, Hutzel Hosp./Wayne State Univ., Detroit, MI

Studies suggest that weight for length (w/l), rather than birthweight percentile (bwt%) may be more sensitive for the identification of symptomatic IUGR. We studied 12,238 non-LGA near term births (≥ 36 wks) to compare the utility of measures of growth (w/l, bwt%, w/l for gestational age and birthweight (bwt)) in the prediction of six adverse growth-related neonatal outcomes. By multivariate analysis, overall morbidity was best predicted by w/l. W/l (not adjusted for gestational age) showed the strongest association with hypoglycemia, low 5 minute apgar score and polycythemia. Only meconium aspiration was better predicted when w/l was adjusted for gestational age (w/l/ga). No significant relation was found between cesarean section for fetal distress or need for resuscitation and any measure of growth. The most significant predictor of 4/6 outcomes was w/l, with only a small amount of additional variance explained by bwt%. These findings are consistent with the supposition that symptomatic IUGR is related to in utero caloric deprivation as reflected in decreased w/l. Since adjustment for gestational age adds little to the prediction of adverse outcomes in the near term infant, initial identification of the high risk neonate can be expediently made in the delivery room by the simple process of assessing neonatal weight and length. Further evaluation of the low w/l near term infant by the pediatrician should include assessment of bwt%.

530 **PREMATURITY AND FETAL GROWTH: 'NORMAL' WEIGHTS BASED ON ABNORMAL PREGNANCIES.** SF Bottoms, IE Zador, and KL Chan. Wayne State Univ., Hutzel Hospital, Detroit, MI.

Currently the same proportion (10%) of preterm and term are classified as SGA. Consequently "normal" birth weight standards for preterm infants are based exclusively on data from abnormal births (prematures). Recent studies suggest that preterm delivery is associated with diminished fetal growth. The purpose of this study is to develop birth weight norms based on the entire population, including the normal undelivered fetus. We studied 4653 consecutive singleton live births having complete ultrasound examinations and delivering at our hospital from 1983-1988, excluding major congenital malformations. Gestational age at time of ultrasound was calculated based on pediatric examination. There was no significant difference between mean EFW and mean birth weight for the 479 premature infants who delivered within 3 days of ultrasound. EFW percentiles based on the entire population were developed for each week from 26-36 weeks. Classification based on these tables according to gestational age at delivery is summarized below.

	Preterm	Term
SGA	100(20.9%)	332(8.0%)
AGA	351(73.3%)	3406(81.6%)
LGA	28(6%)	436(10.4%)

Growth classification of preterm infants based on EFW differed from that of term infants ($p < 0.0001$), and from current birth weight classification ($p < 0.0001$). We conclude that current growth classification systematically underestimates the relative frequency of diminished growth among preterm infants as compared to those delivered at term.

531 FETAL AND NEONATAL HEMATOLOGIC PARAMETERS IN RED CELL ALLOIMMUNIZATION: PREDICTING THE NEED FOR NEONATAL TOP-UP TRANSFUSION. George R. Saade, M.D.[X], Kenneth J. Moise, Jr., M.D., Michael A. Belfort, M.D.[X], Diane Hesketh, R.N.[X], Robert J. Carpenter, Jr., M.D.; Dept. of Ob/Gyn; Baylor College of Medicine; Houston, Tx.

Recently, there has been an increased awareness for the need for top-up transfusion (TUT) in neonates treated with intrauterine transfusion (IUT) for red cell alloimmunization. **Purpose:** To determine whether any fetal or neonatal hematologic parameter can be used to predict the need for TUT. **Material and Methods:** The records of 36 patients who underwent IUT's and had adequate neonatal follow-up were reviewed. The patients that needed TUT were compared to those that did not using X^2 and unpaired Student t test. **Results:** No statistically significant difference was found between the two groups in the number of IUTs or neonatal exchange transfusions, the gestational age at first IUT, the presence or absence of hydrops, the fetal hct or hgb at the last IUT, and the umbilical cord retic count or bilirubin. Fetuses who required TUT had a lower retic count at their last IUT (1.5 ± 2.3 vs 4.9 ± 4.8 %; p = .01) and longer duration between their lowest retic count and delivery (42.1 ± 23.5 vs 24.3 ± 17.6 days; p = .03). In addition, these newborns had a higher umbilical cord hgb (13.5 ± 1.7 vs 11.0 ± 2.3 gm %; p < .01) and % adult red cells (96.0 ± 7.7 vs 82.5 ± 19.6; p = .02). None of the newborns with a cord hgb < 11.8 gm% required TUT. **Conclusions:** The data suggests that the need for TUT is related to the extent and duration of fetal bone marrow suppression caused by transfusion of adult cells in utero.

532 THE RELATIONSHIP OF "COMPLETE" CORD ARTERIAL BLOOD GASES TO GESTATIONAL AGE AND NEONATAL OUTCOME. A. Hiett, L.Devoe A.Youssef, Dept.OBGYN,Med.Col.Georgia, Augusta,GA

We reviewed umbilical artery blood gases(UABGs) in 3000 consecutive deliveries, 32 - 42 weeks' gestation,to determine rates of neonatal metabolic (MET),mixed(MIX),and respiratory (RESP) acidoses, (by criteria of Gilstrap, Obstet Gynecol 1987;70:191), and gestational-age related rates of infant morbidity not due to sequelae of respiratory distress or major anomalies. Acidosis types and rates were similar for term(T) and preterm(PT) groups (v. table, numbers in ( ) = morbidity).

GA(wks)	Total	MET	RESP	MIX
32-36	261	3(1)	17(6)	20(9)
37-42	2315	16(7)	151(26)	233(38)

Morbidity was significantly lower for all T (3%) than PT (6%) acidosis groups. While morbidity rates rose as pH fell (7.20 -> 6.75), 50% of morbid cases in both groups occurred at pH >7.15 and < 7.20. Morbidity in the PT group was similarly distributed in all acidosis types (X^2=.42,NS); in the T group, it was significantly higher with MET (p=.02). These data suggest that UA pH alone may be adequate for preterm infants as any acidosis appears equally harmful. Term gestations require complete UABGs to discriminate risk of morbidity.

533 **INTRAUTERINE GROWTH RETARDATION: 1988 U.S. DATA COMPARED TO PREVIOUS STANDARDS**

M. Kiely,* J.L. Kiely,* Maternal and Child Health Bureau, Health Resources and Services Administration, Rockville, MD, and NCHS.

17 years ago Hoffman et al. published birth weight for gestation percentiles based on a 50% sample of all US live births born in 1968 (Obstet Gynecol Surv 1974;29:651-81). The purpose of our analysis was to explore whether distributions of birth weight for gestation in the US shifted upward between 1968 and 1988. We used US live birth files from the National Center for Health Statistics. For each year from 1968 to 1988, we calculated the median birth weight and the 10th percentile for gestations between 28 and 45 weeks. This was done separately for 8 groups by race (blacks, whites), parity (primiparae, multiparae) and sex. In all 8 race/parity/sex subgroups there were substantial upward shifts in birth weight at gestational ages of 36 weeks and more. The 10th percentile increased 80-190 grams. The table below shows these upward shifts for selected gestational ages among males. These data provide further evidence for the recommendation of Goldenberg et al. (Am J Obstet Gynecol 1989; 161: 271-7) that new national standards for IUGR should be developed.

Tenth percentile birth weight values at various gestational ages				
	36	38	40	42
Whites: 1968	2259	2621	2890	3010
1988	2426	2807	3050	3090
Difference	+167	+186	+160	+80
Blacks: 1968	2227	2544	2676	2660
1988	2325	2665	2835	2835
Difference	+98	+121	+159	+175

534 **INFANT MORTALITY IN HIGHER ORDER MULTIPLE BIRTHS, UNITED STATES 1960 AND 1983-1985.**

J.L. Kiely,* M. Kiely,* J.C. Kleinman,* National Center for Health Statistics, Hyattsville, MD and Maternal and Child Health Bureau, HRSA.

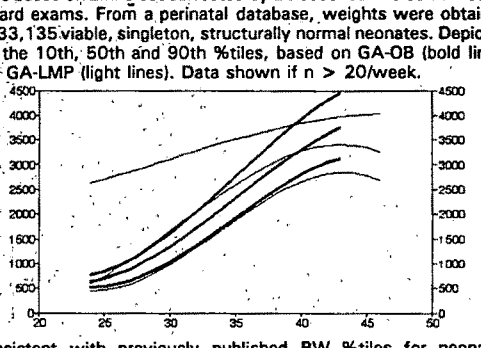
Recent reports on higher order multiple births (triplets & higher) cared for in tertiary centers suggest that their survival has improved dramatically (e.g., Gonen et al. Am J Obstet Gynecol 1990; 162: 454-9). But hospital-based studies may not be generalizable to the entire birth population. We therefore analyzed birthweight-specific infant mortality rates (IMRs) among singletons, twins, and higher order multiple births in the U.S. in 1983-5 and compared the latter rates to those in 1960. In whites in 1983-5, the relative risk (RR) of infant mortality among higher order multiple births compared to singletons was 15.9 (130.3 vs. 8.2 per 1000 live births). In blacks, the RR was 13.2 (224.5 vs. 17.0). This was due almost entirely to the lower weight distribution of higher order multiple births. In whites, 89% weighed <2500g, as compared to 4.8% of singletons. In blacks, 92% weighed <2500g, as compared to 11.4% of singletons. Higher order multiple births who weighed 500-999g had about the same IMR as singletons. In weight categories 1000-2499g, the IMR in higher order multiple births was much lower: weight-specific RRs ranged from 0.30 to 0.73. Between 1960 and 1983-5, in high order multiple births the IMR declined 49% in VLBW white infants (from 683 to 351), 55% in VLBW black infants (from 941 to 423), 80% in whites weighing 1500-2499g (from 75 to 15), and 73% in blacks weighing 1500-2499g (from 129 to 35). Similar patterns were found in analyses of perinatal mortality. Thus, modern intensive care techniques have had a similar beneficial impact on the survival of singletons, twins,* and higher order multiple births.

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* See Kleinman et al. (Am J Epidemiol 1991; 133: 133-43) for a detailed analysis of U.S. time trends in infant mortality in twins and singletons.

535 MENSTRUAL DATING-NOW AN INADEQUATE ESTIMATOR OF GESTATIONAL AGE MP Dombrowski, HM Wolfe, YW Brans,* AA Saleh, RJ Sokol, Depts of Ob/Gyn and Pediatrics, Wayne State Univ./Hutzel Hosp., Detroit, MI

Although current practice is to use fetal ultrasound and Ballard for gestational age (GA) dating, birth weight percentiles (BW %tiles) are still based solely on GA by last menstrual periods (GA-LMP). The purpose of this study was to develop a standard consistent with current technology and practice. Obstetric estimates of GA (GA-OB) were based on LMPs but corrected by ultrasounds and confirmed by Ballard exams. From a perinatal database, weights were obtained for 33,135 viable, singleton, structurally normal neonates. Depicted are the 10th, 50th and 90th %tiles, based on GA-OB (bold lines) and GA-LMP (light lines). Data shown if n > 20/week.



Consistent with previously published BW %tiles for neonates preterm by GA-LMP, data are widely divergent with a decrease in median BW %tiles beyond 42 weeks when compared to BW %tiles by GA-OB. We conclude: 1) use of ultrasound increases the precision of GA dating, 2) BW %tiles based solely on LMP are likely to be inaccurate for preterm and post-term gestations, 3) since fetal growth typically continues to 44 weeks, macrosomia rather than growth retardation is the greater risk of post-datism.

ADDITIONAL ABSTRACTS*

CATEGORIES	ABSTRACT NOS.
Maternal-Fetal Physiology	536-546
Medical Complications of Pregnancy	547-565
Antepartum Fetal Testing	566-574
Clinical/Operative Obstetrics	575-585
OB Anesthesia & Pharmacology	586-588
Genetics and Teratology	589-601
Neonatology	602-604
Computers	605-608
Labor	609
Fetal Therapy	610-611
Diagnostic Ultrasound	612-626
Doppler	627-632
Hypertension	633-639
Infectious Disease	640-647
Prematurity	648-662
Intrapartum Fetal Evaluation	663-664

*The following listing of abstracts are those accepted for this year's meeting, but withdrawn by the authors prior to publication: 538, 541, 542, 550, 556, 560, 567, 570, 573, 575, 579, 582, 586, 596, 598, 599, 603, 605, 610, 614, 615, 617, 619, 626, 631, 632, 635, 643, 646, 661.

536 MATERNAL SERUM ALPHA-FETOPROTEIN LEVELS DO NOT INCREASE WITH LABOR

C. M. Meyers, R. N. Andersen[x], S. Elias, M. Brownlee[x], E. A. Tolley[x], J. L. Simpson, Dept. Ob/Gyn, Univ. of Tennessee, Memphis.

The usefulness of second and third trimester maternal serum alpha-fetoprotein (MSAFP) as a predictor of outcome in complicated pregnancies is under investigation. We sought to clarify the effect of labor, a potentially confounding variable in these studies, on MSAFP. In 13 singleton, term pregnancies without antepartum complications, MSAFP was measured at the following times: (1) prior to labor, (2) on admission for delivery, (3) active labor, (4) during the second stage of labor, (5) after delivery, and (6) the first day post partum. MSAFP levels decreased between samples obtained prior to labor (1) and in labor (2 or 3) (p=0.001), and continued to decrease in subsequent samples. One might have predicted the opposite result, if fetal-maternal transfusion occurs regularly during labor. Conclusions: (1) MSAFP decreases after the onset of labor in comparison to prior samples in uncomplicated, term pregnancies. (2) MSAFP does not increase during labor, suggesting fetal-maternal transfusion during labor is not common in these individuals.

537 LOW FOLATE AND B_{12} LEVELS AND THE INCIDENCE OF SPONTANEOUS ABORTIONS. Ran Neiger, MD, Charlotte Wise, MD[x], Stephen A. Contag, MD, Marea Tumber[x], Jacob A Canick, PhD[x]. Brown University/Women and Infants Hospital, Providence, Rhode Island.

We examined the hypothesis that low folic acid levels around the time of conception were associated with an increased rate of miscarriages. Over a six-months period we obtained folate and B_{12} levels on 225 women who presented to Women and Infants Hospital emergency room due to first trimester vaginal bleeding, and studied their pregnancy outcomes. Inclusion criteria were intrauterine pregnancy of less than 14 weeks gestation and HCG>25 mIU/ml. Seventy-four women were excluded due to low HCG levels, ectopic pregnancies, molar pregnancies, elective terminations, or lack of information about pregnancy outcome. Of the 151 women who were included, 99 had normal folate levels (≥4.0 ng/ml) and 52 had low folate levels. The average age, gravidity, parity, gestational age and HCG levels at the time of presentation were similar between the two groups. The rate of spontaneous abortions was similar among women with low folate compared with those whose folate was normal (39 of 52 (75%) and 66 of 99 (67%), p=0.3). Of the 46 women who delivered a viable newborn, the average gestational age at delivery, birth weights, and 1 and 5 minutes Apgar scores of the two groups were similar. There was no difference in pregnancy outcome among women whose B_{12} levels were low compared with those with normal B_{12} levels. We conclude that among pregnancies complicated by first trimester vaginal bleeding, folate and B_{12} levels appear to have little association with pregnancy outcome. The benefit of pre-pregnancy folic acid supplementation for improving pregnancy outcome awaits further evaluation.

539 MATERNAL CAFFEINE CONSUMPTION AND UMBILICAL ARTERY VELOCIMETRY IN NORMAL THIRD TRIMESTER PREGNANCY. L. Devoe, MD, C. Murray, RN,[x] A.Youssef, MD,[x]. Dept OBGYN, Med.Coll. of Georgia,Augusta, GA.

Most fetuses receive caffeine exposure via maternal ingestion. Since caffeine has vasoactive effects, we sought to determine if its chronic maternal consumption could influence umbilical artery(UA)resistance. We studied UA Doppler velocimetry of 20 normal third trimester fetuses, from 30 to 40 weeks. Serial systolic:diastolic (S:D) ratios of UAs were obtained every 2 weeks. Values from Doppler insonation at 3 different intervals(10 cycles each) were averaged for each session.Using a previously validated questionnaire,10 patients were identified as high caffeine consumers (> 500 mg/day) and 10 as low consumers (< 200 mg/day). Comparison of S:D ratio trends within each group showed no significant time effect(p=.89)or differences between groups (p=.37). Regression plots of maternal plasma caffeine levels at each study vs S:D ratio showed no significant correlation (r=.03, p=.83). The level of maternal caffeine intake does not appear to affect the course of UA Doppler velocimetry observed in normal pregnancy or, by inference, alter fetal umbilical vascular resistance.

540 UMBILICAL CORD γ-GLUTAMYL TRANSFERASE (GGT): IS IT A MARKER FOR FETAL ABNORMALITY? Mordechai Hallak, Stanley M. Berry, Jennifer A. Bichalski,[x] Honor M. Wolf, Mark P. Johnson,[x] Mark I. Evans, David B. Cotton; Dept Ob/Gyn; Wayne State University / Hutzel Hospital; Detroit, MI

Normal values for fetal hematologic parameters, blood chemistries, and liver function tests (LFT's) have been previously established. Elevated GGT is a sensitive marker for hepatic dysfunction even though it is produced by other organs including the pancreas, kidney, and heart. Infection and placental insufficiency have both been associated with elevated fetal GGT levels; hepatic inflammation has been implicated in the former condition and hypoxic hepatocellular damage in the latter. **Material and Methods:** We evaluated LFT's and complete blood counts in 30 consecutive fetal blood specimens obtained by cordocentesis. GGT's were obtained on 25 specimens. The indications for cordocentesis included: fetal malformation (12), red blood cell isoimmunization (6), possible fetal infection (5), oligohydramnios (4), advanced maternal age (1), combined problems (2). Mean gestational age was 26.8 ± 5.0 weeks (range of 19 - 37). **Results:** All fetal hematologic parameters were within normal limits. LFT results were as follows (mean ± SD):

LFT's	Results	Normal Levels
Total protein (g/dl)	3.58 ± 0.78	3.04 ± 0.06
Albumin (g/dl)	2.17 ± 0.42	2.14 ± 0.04
Total bilirubin (mg/dl)	1.63 ± 0.33	1.57 ± 0.06
ALT (GPT) (IU/l)	8.51 ± 4.24	not reported
AST (GOT) (IU/l)	25.23 ± 8.04	21.1 ± 2.0
GGT (IU/l)	138.08 ± 102.6*	24.4 ± 9.6

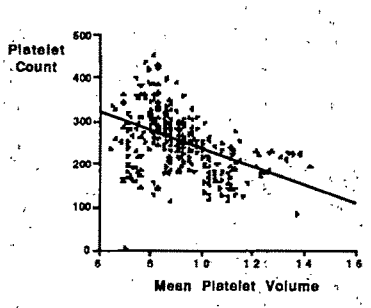
Conclusions: 1. Fetal GGT levels are significantly ($p < 0.001$) elevated in several abnormal fetal conditions. 2. These data suggest that several seemingly unrelated fetal conditions are associated with mild degrees of liver dysfunction; alternatively, GGT elevations in these circumstances may be related to other organ systems.

543 **RELATION OF TOTAL MATERNAL WATER (TMW), ARTERIAL PRESSURE (AP) AND PROGESTERONE (PROG) TO NEWBORN WEIGHT (BW).** F Mardones-Santander[x], G Salazar[x], F Mardones-Restat[x], J Alvear[x], GJ Valenzuela. INTA, U. Chile, Santiago and Ob Gyn, Loma Linda U., Santiago and California.

Maternal factors that influence have been studied separately, and usually in small samples. We decided to assess the relation of TMW, maternal weight, ponderal index, plasma volume, AP, Hcto, PROG, aldosterone and estradiol to BW. We determined those parameters in a total of 114 normal pregnant women, with a wide range of maternal weights, during the last part of pregnancy. Mean ± SD were TMW= 33.08± 5.5, PROG 182 ± 68.7 ng/ ml, sytolic AP 113± 6.4 and diastolic AP 69.07 ± 8.6 mm Hg. The data was analyzed by logistic regression. BW was positively correlated to TMW ($r=0.31$), PROG ($r=.19$) and AP ($r=.26$). Other classical factors (maternal weight, etc.) became important only when the effect of TMW was removed from the analysis. We concluded that the normal mechanism that produce maternal water retention is important in determining BW. The exact mechanism of how these factors influence BW remains unclear at this time.

544 A COMPARISON OF THE CHANGES IN PLATELET SIZE AND PLATELET COUNT IN PREECLAMPSIA. J J Walker, A D Cameron, C Singer[+], C Fraser[+]. Perinatal Research Unit, Glasgow Royal Maternity Hospital, Glasgow, Scotland, UK.

Platelet count is known to fall in preeclampsia. We have previously shown that rising platelet size may precede the drop in platelet count. The purpose of this study was to investigate the relationship between platelet count and platelet size in patients who were preeclamptic. Three hundred and twenty six primigravid patients with moderate or severe preeclampsia were studied. Blood was drawn and the platelet count and mean platelet volume were calculated using a Coulter Counter S.



There was a significant negative correlation between the platelet count and the mean platelet volume ($r=-0.5$, $p<0.001$). **Conclusions.** These results imply that changes in platelet size is associated with a fall in platelet count. However, the count will often remain in the normal range.

545 **MATERNAL FETAL GLUCOSE GRADIENT.** R.Shiffman, G.Kuo[x], J.Josimovich[x], Dept. Ob/Gyn, Methodist and Brookdale Hospa., B'klyn, NY

Maternal glucose (MG) is thought to cross the placenta by facilitated diffusion. Evaluation of maternal-fetal glucose gradients (Delta) was done at term. **METHODS:** 74 patients with singleton pregnancies and no known diabetes had MG and cord venous and arterial glucose (VG,AG) drawn at delivery. Delta (MG-VG) was calculated as well as FDelta (VG-AG). Correlation coefficient and t-tests were performed using the SPSS program. **RESULTS:** Pearson r and P values are listed below. When comparing MG≤120 to MG>120, there was a highly significant difference. No correlation was found with neonatal weight.

	MG/VG	MG/AG	MG/Delta	MG/FDelta
r:	.8626*	.7468*	.5261*	.4034* *P<.001
	VG	AG	Delta	FDelta
MG≤120:	72.9±1.4	63.2±1.9	17.1±1.1	1.7±1.2
MG>120:	107.5±2.8*	89.0±4*	27.5±3.7*	18.5±1.7*

CONCLUSIONS: At higher MG levels there is a significantly greater Delta, suggesting a placental regulatory mechanism for varying glucose delivery to the fetus. Similarly, the amount of glucose utilized by the fetus (proportional to Fdelta) may also depend on maternal levels.

546 EFFICACY OF PREINDUCTION "DILAPAN" ON LOWERING THE CESAREAN SECTION RATE. GJ Gilson, JF Smith, LB Curet, LA Izquierdo, MS Chatterjee, GM Joffe[x], GO Del Valle[x]. University of New Mexico Hospital, Albuquerque, New Mexico.

The objective of the current study was to investigate whether or not "DILAPAN" (polyacrylate hydrogel) intracervical hydroscopic dilators (DIL) would have an effect on the outcome of oxytocin induction of labor at term. Methodology entailed study of 59 term gravidas randomized to receive preinduction DIL or no pretreatment. All subjects had Bishop scores of 4 or less. Nulliparas and multiparas were equally represented in the two groups. Results revealed that, compared to controls, the DIL group exhibited a significant change in Bishop scores (2.8±1.0 to 5.0±2.0, $p\leq .0001$), but no significant difference in induction to delivery interval (DIL:19.2±9.7 hours, control: 14.9±5.6 hours). Of more importance, there was no significant difference in cesarean section rate (DIL:13 of 29[44.8%], control: 8 of 30[26.7%]), although there was a tendency for DIL subjects to have a more advanced dilatation when they underwent abdominal delivery. Infant weights (DIL: 3118±721, control: 2981±713) and Apgars (DIL:8.6±0.9, control: 8.5±0.8) were not significantly different and no adverse maternal or fetal effects could be attributed to use of the device. Conclusion: Preinduction cervical ripening with DIL does not appear to appreciably lower the cesarean section rate.

547 **RISK FACTORS FOR THE RECURRENCE OF GESTATIONAL DIABETES** F.L. Gaudier[x], M.G. Poist[x], J.C. Hauth, D. Corbett[x]
The University of Alabama Hospitals, Birmingham

We evaluated the recurrence of gestational diabetes mellitus (GDM) by identifying ninety women with a pregnancy complicated by glucose intolerance and whose subsequent pregnancy was managed at our institution. Forty-seven (52%) of the patients had a recurrence of GDM in their subsequent gestation.

	Recurrent (n=47)	Non-Recurrent (n=43)	P
Race (Black)	79%	84%	0.55
Family History	57.4%	46.5%	0.30
Index Pregnancy			
Macrosomia (≥4000g)	23%	7.5%	0.05
Glucose values (mg/dl)			
Screening Test (1 hr value plasma)	189±50	168±39	0.04
GTT - Fasting	110±25	99±22	0.42
1 hour	228±43	205±35	0.01
2 hour	225±60	184±38	0.0004
3 hour	163±54	158±42	0.63
Required Insulin	69%	31%	0.04
Subsequent pregnancy			
BMI ≥35	34.1%	10.0%	0.01
Pre-pg wt. (kg)	83.4±23.2	75.0±22.9	0.09
Wt. gain in pg (kg)	10.9±5.7	13.1±7.0	0.11
Newborn wt. (gm)	3479±732	3359±680	0.42

BMI = Body Mass Index

We conclude that women with a prior history of GDM are at increased risk for recurrence. These patients may benefit from earlier screening for glucose intolerance in their subsequent pregnancies and especially those who are obese, had fetal macrosomia, or required insulin during their previous pregnancies.

548 HbIAc PREDICTS PREGNANCY MORBIDITY IN DIABETICS. R. Figueroa, U. Verma, F. Wiltshire[x], N. Tejani.
Dept. of Ob/Gyn, NY Med. Coll., Valhalla, NY.

Objective To evaluate the correlation on initial HbAlc and adverse outcome in diabetic pregnancies. **Study design** Medical records of 174 pregnancies in diabetic women were reviewed. Information obtained was initial HbAlc value and gestational age (≤12 wks., ≤20 wks., ≤24 wks., >24 wks) when obtained, adverse pregnancy outcome (major congenital malformations, spontaneous abortion, fetal death after viability) and normal outcome. Pregnancies were subdivided into HbAlc ≤9%, HbAlc >9% -≤12% and HbAlc >12%. Data was analyzed using ANOVA and t-tests. **Results** Compared to pregnancies with normal outcome, HbAlc was higher in pregnancies with major congenital malformations (10.2% vs 7.3%; p<0.01), spontaneous abortions (13.3% vs 7.3%; p<.005), and when all adverse outcomes were considered (10.9% vs 7.3%, p<.005). A HbAlc of >12% at ≤12 wks. gestation predicted a 100% morbidity.

TABLE
ADVERSE PREGNANCY OUTCOME (CUMULATIVE NUM.)

HbAlc	GA (Weeks) ≤12	≤20	≤24	ALL
>12%	6/6	10/11	11/13	11/16
>9% ≤12%	2/8	3/17	5/20	7/27
≤9%	0/6	2/18	3/27	11/131

Conclusions HbAlc is a reliable predictor of adverse pregnancy outcome.

549 **FIXED MINIDOSE WARFARIN FOR PROPHYLAXIS OF THROMBOEMBOLIC DISEASE IN PREGNANCY: A SAFE ALTERNATIVE FOR THE FETUS?** Robert S. McDuffie, Jr., M.D., Sanford Peck, M.D.[x], Richard P. Porreco, M.D., Presbyterian/St. Luke's Perinatal Program, University of Colorado Health Sciences Center, Denver, Colorado

Fixed minidose warfarin may be effective prophylaxis for venous thrombosis in high risk patients. Complete anticoagulation with warfarin in the second and third trimester of pregnancy may lead to fetal and maternal bleeding complications. Parenteral heparin by subcutaneous injection or infusion pump is inconvenient, painful and associated with complications of bleeding, thrombocytopenia, and osteoporosis. The following patient offered the opportunity to study the usefulness and safety of minidose warfarin in late pregnancy.

Case Report: A 28 year old para 1 with antithrombin III deficiency suffered a right subclavian vein thrombosis at 18 weeks gestation and was treated with intravenous heparin with resolution. Subcutaneous heparin was substituted for prophylactic therapy but was unsuccessful in prolonging the partial thromboplastic time (PTT) to any degree. A continuous infusion pump was required to assure adequate prophylaxis, but the patient was unreceptive to prolonged therapy of this type. Minidose warfarin (1 mg a day) was offered as an alternative at 32 weeks gestation. Maternal and fetal blood samples were analysed at 33 and 36 weeks gestation.

	33 Week Maternal	33 Week Fetal	36 Week Maternal	36 Week Fetal
PT	12.3 sec.	14.3 sec.	12.3 sec.	13.4 sec.
II	87%	23%	91%	24%
VII	159%	48%	195%	52%
IX	115%	15%	194%	15%
X	121%	22%	152%	37%
Sonoclot	"hyper"	"normal"	"normal"	"normal"
Warfarin	1.0 mcg/ml	0.09mcg/ml	<0.1mcg/ml	<0.1mcg/ml

Conclusion: Fixed minidose warfarin did not result in any clinically significant abnormalities of maternal-fetal coagulation. Some vitamin K-dependent factors in the fetus were mildly depressed. Efficacy of minidose warfarin in pregnancy requires further investigation, though this case study suggests that the fetus is not at increased risk of hemorrhage.

551 LONG-TERM HEALTH OF CHILDREN OF INSULIN DEPENDENT WOMEN

J.E. Converse[x], Dept. of OB/GYN, University of Wisconsin, Madison, WI, M.S. Cranley[x], School of Nursing, University of Buffalo, Buffalo, NY, and L.B. Curet, Dept. of OB/GYN, University of New Mexico, Albuquerque, NM

A retrospective, descriptive study was designed to investigate the health status of children born to insulin dependent diabetic mothers (IDDM). The relationship of the child's health to the maternal obstetrical course was also examined. The convenience sample consisted of 80 children born to 56 predominately married, middle-class, medically insured IDDM mothers who received obstetrical services from a midwestern university perinatal center from the years of 1971 to 1987. The children ranged in age from 7 months to 16 years. Three children had died, 2 in infancy and 1 in childhood.

Conclusion: The results showed that in comparison to general population national health statistics, the children in this study had two to four times greater incidences of child health conditions related to medical, neurological, and developmental problems at birth, in the neonatal period, and throughout childhood. The greater the number of maternal health risk factors, the earlier the infant was born, and the more health problems the child had at birth and in childhood. The differences in child health were not related to the trimester in which prenatal care began with the perinatal program nor correlated with maternal hyperglycemia and elevated glycosylated hemoglobin.

552 MANAGEMENT OF PREMATURE RUPTURE OF MEMBRANES (PROM) : STATE OF THE ART, 1991, Uchenna Nwosu, M.D. Dept. of OB/GYN, East Tennessee State University College of Medicine, Johnson City, TN.

We surveyed all 1,041 members of the Society of Perinatal Obstetricians by signed questionnaire concerning their current practice in the management of PROM at various gestational periods, with regard to induction of labor, use of antibiotics, tocolytic agents and corticosteroids.We received 529 replies, 235 from regular and 294 from associate members.Analysis showed no difference in the practice of the two groups.With respect to labor induction the most controversial periods were 33-35 weeks with mature fetal lungs where 50% will induce from 0-48 hours and 50% will await spontaneous onset of labor, and 19-22 weeks where 48% remained silent.Most responders do not use antibiotics at any time, and most of the antibiotic users do so for a limited period of time only, with ampicillin the overwhelming choice.Most responders do not use tocolytic agents at any time and most users do so therapeutically rather than preemptively, with $MgSO_4$ favored 2:1.Most responders do not use cortiosteroids to enhance fetal lung maturity at any period. This survey indicates need for a study of outcome of newborns induced with mature lungs 0-48 hours following PROM at 33-35 weeks, as compared with similar newborns delivered following spontaneous labor.

553 PERINATAL OUTCOME IN DIABETIC PATIENTS WITH NEPHROPATHY AND RETINOPATHY VS. DIABETICS WITH ISOLATED PROLIFERATIVE RETINOPATHY. Joffe GM, Del Valle GO, Izquierdo LA, Vill M[x], Jones D[x], Gilson GJ, Chatterjee S, and Curet LB, Univ. New Mexico Med. Ctr., Albuquerque, NM

An eight year review of pregnant diabetic patients with nephropathy and retinopathy vs those with isolated retinopathy was completed. Out of 10,500 admissions, 14 patients with both nephropathy and retinopathy and 9 patients with isolated proliferative retinopathy were identified (incidence 0.13% and 0.08% respectively). Patients with nephropathy and retinopathy were older (26.4+/-4.43 vs 21.8+/-2.28 years p=.005), had higher incidence of chronic hypertension (86% vs 0% p=.001), had higher incidence of cesarean section (86% vs 25% p=.01), had earlier gestational age at delivery (34.4+/-2.6 vs 36.8+/-2.41 wks p=.01), and had lower neonatal birth weight (2388+/-785 vs 3214+/-863 gm p=.01). Maternal age of onset of diabetes, gravidity, parity, Hgb A1C, APGAR scores, and incidence of congenital anomaly (12.5% in isolated retinopathy group) were not significantly different. This study demonstrates that isolated proliferative retinopathy may precede development of nephropathy and chronic hypertension and is associated with significantly better perinatal outcome.

554 EFFECT OF GENDER ON PERINATAL OUTCOME IN PREGNANCIES COMPLICATED BY DIABETES.
L.A. Bracero, and, S. Cassidy*, Dept. of OB/GYN New York Medical College, Valhalla, New York

Low birthweight female infants have been shown to have a higher survival rate than low birthweight males. This sex difference in mortality has been attributed to a higher incidence and severity of Respiratory Distress Syndrome in male infants. The purpose of this study was to determine whether there is a sex difference in the morbidity and mortality of infants born to diabetic mothers. A review of 107 newborns from diabetic mothers was performed. There were 63 males and 44 female infants. We looked at demographics, parity, White's classification, glycemic control, blood pressure, Hgb/Hct, mode of delivery, incidence of low birthweight & preterm deliveries and found no statistically significant difference between the groups. There was one female stillbirth as a result of an episode of ketoacidosis in the mother. There was more morbidity in the male group mainly as the result of hypoglycemia (23.8% of males vs 6.8% of females; Relative Risk=3.50; P value=.0208) and need to stay in the NICU $\geq$ 2 days (52.4% of males vs 29.5% of females; Relative Risk =1.78; P value=.0189). It appears that there is a disadvantage to being the male infant of a diabetic mother.

555 OBSTRUCTIVE UROPATHY: A CAUSE OF REVERSIBLE HYPERTENSION IN PREGNANCY. A.J. Satin[x], G.L. Seiken[x], F.G. Cunningham. Dept. OB/GYN, Univ. Texas Southwestern Med. Ctr., Dallas, TX and Dept. Nephrology, Brooke Army Med. Ctr., San Antonio, TX.

Hypertension with deterioration of renal function after mid-pregnancy often signifies preeclampsia and the need for delivery. Over the past 12 years, we have encountered four pregnant women with reversible hypertension related to obstructive uropathy. These women presented between 24 and 35 weeks gestation with mean arterial pressure increased >20 mmHg above pregnancy baseline accompanied by significantly increased serum creatinine (mean increase = 2.5mg/dl). Although pregnancy-induced hypertension was considered initially in all, there was no other evidence for preeclampsia. Ureteral obstruction was confirmed by ultrasound and was associated with congenital urinary anomalies in two, massive leiomyoma in one, and hydramnios in the other. Relief of obstruction by ureteral stent placement or decompression of amnionic fluid volume resulted in resolution of hypertension and a fall in serum creatinine (mean decrease = 2.2mg/dl). Despite this, all four developed recurrent hypertension and/or renal insufficiency within 1 to 7 weeks, necessitating delivery between 31 and 36 weeks gestation. Importantly, delivery was delayed more than 6 weeks in the 3 women in whom stents were placed. We can implicate urinary obstruction as the cause of hypertension because blood pressure control improved after relief of obstruction. In one early report (NEJM, 278:1133,1968) unilateral hydronephrosis was associated with renin mediated hypertension. Studies in men have implicated bilateral ureteral obstruction as a cause of hypertension secondary to salt and water retention. Thus, urinary obstruction has been reported as a cause of reversible hypertension in nonpregnant patients, but to our knowledge, this is the first report of ureteral obstruction with reversible hypertension secondary to the gravid uterus.

557 **A NOVEL THERAPEUTIC APPROACH FOR REFRACTORY HYPEREMESIS GRAVIDARUM**

W Burrows, S Zwick[x], MA Krew, L Dierker, PM Catalano, MetroHealth Medical Center, Case Western Reserve University, Cleveland, Ohio

A two year retrospective analysis was done to evaluate the use of nasoalimentary feeding (Dobhoff tubes) in the therapy of severe hyperemesis gravidarum. At our institution, after an initial attempt to treat hyperemesis on an outpatient basis, failures are admitted for prolonged intravenous hydration and antiemetic therapy. Severe cases were unresponsive to this treatment or required multiple admissions. The charts of 55 patients with an admission diagnosis of hyperemesis gravidarum were reviewed. Seven (13%) received nasoalimentary tube feedings. These 7 included patients with prior pregnancies involving intractable hyperemesis (5), prior elective abortion for hyperemesis (3), multiple admissions for hyperemesis in the index pregnancy (5) and prolonged admission over 7 days (7). These 7 patients had a mean of two admissions prior to initiation of tube feedings. Five cases were considered to be successfully treated for intractable hyperemesis. Two cases were considered treatment failures. In one, the patient was unable to tolerate continued tube feedings, (but had no further hyperemesis). In the other case, symptom relief was transient with multiple subsequent admissions for hyperemesis. In all but this case, no further admissions were required after nasoalimentary feeding began. Two patients continued nasoalimentary therapy after discharge.One patient receiving nasoalimentry therapy electively aborted for a fetal anomaly (holoprosencephaly). Of the 48 who were treated in the standard fashion, 4 (8%) aborted because of hyperemesis. A prospective randomized study is being implemented to evaluate further this low cost alternative to central hyperalimentation.

558 DEPRESSIVE MOOD AT THE BEGINNING OF PREGNANCY

L.Duperron,[x] J.F.Saucier,[x] H.David,[x] Dept. Ob/Gyn, Psychiatry Sainte-Justine Hospital, Montreal, Canada.

The goal of the study was to determine whether there are specific factors which identify patients at increased risk for depression in early pregnancy. Four hundred and twelve primiparous (56.6%) and secundiparous (43.4%) were interviewed in early pregnancy, between the 10th and the 22nd week. They were from all social classes; 55.4% (N: 229) were married, 31.2% (N: 128) were in a stable relationship, 6.1% (N: 25) were single, 1.5% (N: 6) were separated or divorced, 1.5% (N: 6) were separated or divorced and remarried, and 4.4% (N: 18) were separated or divorced and living in a stable relationship. No relationship was found between the social class or the civil status and depressive mood, as measured by the short form of the Beck scale. On the other hand, the following situations in these pregnant women were significantly related to the presence of depressive mood: 1- Having a male child (among secundiparous subjects). 2- Being 35 to 39 of age (as compared with being 20 to 34). 3- Having a weak support system, especially if one finds it unsatisfactory. 4- Having two disturbing persons in their social environment who cannot be avoided. In addition, women whose parents were divorced when they were a child or a teenager, were more often inclined (p.07) to feel depressed when pregnant. Ongoing studies will determine whether these factors identify patients at increased risk of post-partum depression.

559 **SARCOIDOSIS IN PREGNANCY**

J.C. King, Dept. Ob/Gyn, Georgetown University School of Medicine, Washington.DC

Pulmonary sarcoid is a rare complication affecting at most 0.05% of pregnancies. Previously it has been suggested that no special management during pregnancy was necessary for patients with sarcoid since clinical status is rarely changed. We report 6 cases of advanced sarcoidosis occurring between 1985 and 1991 complicating pregnancy. Two patients having undergone pulmonary resections for progressive cavitary disease with one of these patients subsequently developing HIV infection potentially from blood transfusion at the time of surgery. Serial pulmonary function testing revealed reduced vital capacity for all patients. Additionally, there frequently was a significant impairment of diffusing capacity. While the use of steroids should not be withheld, 50% of steroid users developed carbohydrate intolerance requiring either diet or insulin therapy. There were no maternal deaths but there was 100% maternal morbidity from a high frequency of infection with staphylococcus aureus or other pathologic organisms. There was a 16% incidence of perinatal mortality(stillbirth) with a higher than expected frequency of smaller infants. The finding of secondary pulmonary hypertension in two patients underscores the importance of serial cardiovascular assessment. In both patients the development of pulmonary hypertension was not discovered until the middle of the third trimester. While physical examination is often suggestive of pulmonary hypertension, the utility of echocardiography and right heart catheterization will be discussed. A clinical management scheme will be presented.

561 **MILD GLUCOSE INTOLERANCE AND PERINATAL OUTCOME**

S. Coleman[x], B. Campbell, P. Roussis, G. Harbison[x], S. Cox
University of Kentucky, Lexington, KY

This retrospective review was to determine pregnancy complications associated with mild glucose intolerance defined by an abnormal 50 gram glucose screen but a normal 3 hour oral glucose tolerance test (OGTT). During a 34 month period, 822 glucose screens were positive (one hour glucose $\geq$140 mg/dl). Of these, 594 (72%) had a normal OGTT, 108 (13%) had one abnormal value, and 120 (15%) were found to have two or more abnormal values (ie. gestational DM). The study consists of 200 normal controls and 100 patients from each group from which delivery data was available. There was no difference in gestational age at delivery, five minute Apgar scores, or shoulder dystocia (Table). Although the incidence of macrosomia in the normal group (10%) was higher than expected, patients with one abnormal value of the OGTT had a significantly higher risk (19%, $P<0.01$) as did those with two abnormal values (16%, $P<0.05$). The incidence of cesarean section was also significantly higher in these two groups of patients. The results suggest that women with mild glucose intolerance (as evidenced by one abnormal OGTT value) are susceptible to the same pregnancy complications as those with gestational diabetes. These pregnancies have a two-fold increase risk of fetal macrosomia and cesarean section when compared to normal controls. Therefore, it would seem reasonable to incorporate a more aggressive approach to mild glucose intolerance in an attempt to further decrease perinatal morbidity. Patients with an abnormal screen followed by a normal 3 hour OGTT were also noted to have an increase in incidence of macrosomia. This could represent false negative oral glucose tolerance tests and warrants further study to evaluate perinatal morbidity and its prevention in this group.

Table I

	Control N = 200	Nl 3° OGTT N = 100	1 Abnl. N = 100	Gest. D.M. N=100
Macrosomia	19 (9.5%)	13	19**	16*
Cesarean section	24 (12%)	11	20+	31***
Forceps/Vacuum	8 (4%)	11	10	5
Preeclampsia	12 (6%)	7	6	3

* $P<0.05$ ** $P<0.01$ *** $P<0.001$ +p = 0.09

562 NEUROFIBROMATOSIS IN PREGNANCY

A. Weissman,[X] P. Jakobi,[X] I. Zaidise,[X] A. Drugan[X]
Dept. Ob/Gyn, Rambam Medical Center and Faculty of Medicine, Technion, Haifa, Israel.

Neurofibromatosis (NF) is one of the most frequent human genetic disorders. Pregnancy in NF patients is rare and commonly associated with a dismal outcome. We report our experience with 34 pregnancies in 9 NF patients treated at our Center (see Table). Despite previous reports, fertility is not impaired in these patients, although they experience higher rates of first trimester abortion (20.7%), stillbirth (8.7%), and IUGR (13%). Cesarean delivery was effected in 26% of these pregnancies. However, with proper prenatal care, it appears that more than 90% of pregnancies which continue beyond the first trimester end in viable newborns.

Table - Obstetric Data in NF Patients

Pregnancies	Abortions		Deliveries**	
	TOP*	Spontaneous	Vaginal	CS
34	5	6 (20.7%)	17	6

* TOP - Voluntary Termination of Pregnancy

** 2/23 fetuses were delivered as stillbirths

563 **DDAVP IN THE MANAGEMENT OF VON WILLEBRAND'S DISEASE IN PREGNANCY.** Swanbeck J*,Baxi L, Hurlet AM*.Dept. Ob/Gyn, Ped. Hem.,College of P & S, Columbia Univ.,Columbia Presbyterian Med. Center.New York,NY

Two patients (3 pregnancies) with Type I Von Willebrands disease (VWD) with previous significant bleeding episodes, were administered DDAVP (1-disamino-8-D arginine vasopressin) .3ugm/kgm at delivery; one of them for delayed post-partum bleeding as well. Factor VIII levels and VW Factor antigen (VWF:ag) level increased throughout pregnancy, and increased acutely following the administration of DDAVP. Intrapartum blood loss was normal for both patients and no untoward effects of DDAVP were noted. Presented below are data from one patient.

Date	Factor VIII 50-150%	VWF:ag 50-150%	Comments
9/8/89	23%	10%	3rd trimester
11/30/89	60%	27%	at delivery pre DDAVP
11/30/89	190%	55%	at delivery post DDAVP

By accurate prenatal diagnosis of VWD Type and by assay of levels of Factor VIII and VWF:ag an assessment of probability of significant bleeding episodes at parturition can be made, particularly in patients with previous episodes of bleeding diatheses. We conclude that timely prophylactic therapy with DDAVP in patients with Type I VWD can acutely raise levels of Factor VIII and VWF ag and avert acute episodes of bleeding at parturition. This treatment avoids the potential complications associated with human blood products. Potential problems (water intoxication, convulsions), from DDAVP administration during pregnancy noted in previous reports were not observed in our patients. Another patient with VWD is now being prospectively managed under the same protocol.

564 **IMPACT OF A DIABETES NURSE SPECIALIST IN A HEALTH MAINTENANCE ORGANIZATION.** J.L. Davis, B.R. Morgan,[X] J.S. Greenspoon, K. Bloumofe,[X] F. Wall,[X] Dept OB-GYN, Cedars-Sinai Medical Center, Los Angeles, Ca.

We demonstrate the benefits of employing a diabetes nurse specialist (DNS) to provide out-patient management and education in a health maintenance organization. Pregnant diabetic patients requiring insulin who were managed by a DNS during Jan-July 1991 were compared to a similar group delivered in 1989-1990 managed without a DNS. Protocols for care were identical between the two groups, except that the DNS did not admit patients for control or education unless out-patient efforts failed. Maternal and neonatal outcomes and costs were compared (Table). Maternal complications consisted of preterm labor, preeclampsia, and infection. Neonatal complications consisted of RDS & metabolic disorders. There was no difference in outcome, but a considerable savings in hospital days.

Table	Before DNS (N=40)	After DNS (N=22)	P*
Mean mat age (yrs)	31.8±6.23	31.6±6.54	NS
#Gest. diabetics (A2)	65.0%	54.5%	NS
#Pregest. diabetics	35.0%	45.5%	NS
Maternal Complications	30%	27.3%	NS
Neonatal Complications	41.5%	47.8%	NS
Primary C section	21%	23%	NS
Gest. age at delivery (wks)	37.4±2.03	37.0±4.04	NS
Birthwt. (gms)	3342±646	3324±955	NS
#Hospital days	9.1±10.7	4.9±7.4	.0004

*NS = not significant
Values are means ± standard deviations or percentages.

565 DOES A HISTORY OF A SEVERELY AFFECTED ANTI-D ALLOIMMUNIZED PREGNANCY PREDICT FUTURE OUTCOME? D.E. Carlson, L.D.Platt, A.L.Medearis, USC+LAC Women's Hospital and Cedars-Sinai Medical Center, Depts. Ob/Gyn, Los Angeles, CA

It is commonly stated that a pregnancy complicated by anti-D alloimmunization will subsequently have increasingly severely affected fetuses. To test this hypothesis we reviewed eight anti-D alloimmunizaed patients who had: 1.history of a hydropic fetus or newborn who either died (IUFD/NND), required intrauterine transfusions (IUT) or multiple (greater than five) newborn exchange transfusions (XEch); 2. with the same partner had two subsequent pregnancies (A/B) under our outpatient management from 1986 to 1990 at USC/LAC; 3. delivered Rh+ newborns with postive direct Coombs on cord blood.

Pt	Hx	APtx	DHct	HBili	NBTx	Outcome
		A/B	A/B	A/B	A/B	A/B
#1	NND	N/1IUT	31/26	19/21	2Ech/6Ech	DW/DW
#2	XEch	N/5IUT	41/34	18/13	Bil/Bil	DW/DW
#3	XEch	N/N	34/41	11/16	Bil/Bil	DW/DW
#4	IUFD	N/N	46/58	9/7	Bil/Bil	DW/DW
#5	IUFD	N/N	48/60	9/20	N/Bil	DW/DW
#6	NND	2IUT/N	-/48	-/12	-/Bil	IUFD/DW
#7	XEch	N/N	48/45	21/13	Bil/Bil	DW/DW
#8	XEch	N/N	26/45	24/14	5Ech/Bil	DW/DW

Key: NND: Neonatal death IUFD: intrauterine fetal demise N:None APtx:antepartum therapy NBTx: Newborn therapy DHct:delivery hematocrit HBili: Highest bilirubin Bil:Bililites DW:Doingwell None of the patients had a predictive pattern of worsening outcomes. We conclude that one cannot counsel patients concerning future pregnancy outcomes based on previous poor history.

566 ANTEPARTUM FETAL HEART VARIABLE DECELERATIONS: WHEN ARE THEY SIGNIFICANT?

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The significance of antepartum fetal heart variable decelerations in pregnancies not at risk for oligohydramnios is uncertain. METHODS: A retrospective review of 2360 nonstress tests was performed. Patients with identifiable risk factors for oligohydramnios (i.e. chronic hypertension, IUGR, post dates) were excluded. Variable decelerations (> 15 bpm, > 15 sec but < 60 sec) were identified in 50 patients and a 4 quadrant amniotic fluid index was obtained. RESULTS: Selected pregnancy outcomes from these 90 patients are presented in Table I.

Table I

	Amniotic Fluid Index		
	< 10 cm	10-20 cm	> 20 cm
n=	23	60	7
intrapartum variables	4 (18%)	9 (15%)	1 (14%)
operative delivery (fetal distress)	2 (9%)	8 (13%)	1 (14%)
meconium	4 (18%)	12 (20%)	1 (14%)
Apgar <7 @ 5 minutes	2 (9%)	0	1 (14%)

*Includes four patients with an AFI < 5 cm.

These results suggest there are no increased incidence of intrapartum complications based on the presence of antepartum variable decelerations. In 12 patients variable decelerations were noted on serial NSTs and they too had no increase in complications. We next evaluated selected pregnancy outcomes based on the indication for obtaining the NST (Table II).

Table II

	Indication		
	Med Comp	Post term (41 wk)	Other
n=	43	15	38
intrapartum variables	7(16%)	4(27%)	3(8%)
operative delivery (fetal distress)	5(12%)	2(13%)	4(10%)
meconium	8(19%)	4(27%)	5(13%)
Apgar <7 @ 5 minutes	2	0	1

Examination of these data reveals an anticipated increased incidence of meconium stained fluid and intrapartum variable decelerations in post term pregnancies. SUMMARY: We conclude from this study that in pregnancies without risk factors for oligohydramnios antepartum variable decelerations are not a poor prognostic sign and do not signal the need for further antenatal testing beyond an AFI.

568 EVALUATION OF INDIVIDUAL DIFFERENCES IN THE RATE OF FETAL HABITUATION.

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The demonstration of habituation in the human fetus suggests that some degree of learning can be assessed early in life. A measure of habituation is the trials-to-criterion (TTC), which is usually arbitrarily defined as the number of trials until "no fetal response occurs for 2-5 consecutive trials." Purpose: To establish an objective basis for specifying the fetal TTC. Seventy human fetuses at 34-40 weeks received 8 trials of a 1-sec vibro-acoustic stimulus (VAS) with a 10-sec inter-stimulus interval. A behavioral response score (BRS) was determined for each trial based on the intensity of the response (10=most intense). By calculating the ratio of the observed response to what would occur by chance, a significant behavioral change was found to occurr between a BRS of 6-4 and a BRS of 3-0; there was no difference in this ratio for a BRS of 3-0, implying that a score of "3" was behaviorally equivalent to "no response." Once a BRS ≤ 3 was achieved, the BRS in 15% of subsequent trials was > 3; this frequency decreased to <5% if the BRS remained ≤ 3 for either 2, 3, or 4 consecutive trials. Furthermore, following a BRS ≤ 3, 35% of the BRS > 3 occurred in runs of ≥ 2; conversely, following a sequence in which the BRS remained ≤ 3 for ≥ 2 consecutive trials, 85% of the responses with a BRS > 3 occurred as a single, isolated event. Using as a TTC a "BRS ≤ 3 for ≥ 2 trials", 70% of the fetuses habituated by trial 8; conversely, > 85% required >8 trials to habituate using the criteria "no response for ≥ 2 trials." Conclusion: a BRS ≤ 3 for ≥ 2 consecutive trials is a more objective criteria for assessing individual differences in the rate of fetal habituation.

569 HEART RATE AND EYE MOVEMENT ORGANIZATION IN THE HUMAN FETUS AT 38-40 WEEKS.

LJ Groome, KP Singh[x], SL Burgard[x], CL Neely[x], AA Bartolucci[x]. Departments of OB/GYN at the University of Alabama at Birmingham, Alabama and the University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Abnormal state organization has been demonstrated in hydrocephalic and growth-retarded fetuses, and in fetuses of diabetic mothers. However, no measure exists to evaluate state organization independent of behavioral state identification. Purpose: to compare two methods for assessing fetal heart rate (FHR) - fetal eye movement (FEM) synchronization: 1) periods of high (HV) and low (LV) variability in FHR and the presence (EM) and absence (NEM) of FEM were determined visually, and the association between FHR and FEM was expressed as the percent study time for which no periods of LV-NEM and HV-EM were identified; and 2) the maximum cross-correlation coefficient (r_{max}) and the lag at r_{max} (L_{max}) were determined by computer analysis of FHR and FEM data. Twenty fetuses at 38-40 weeks were examined for a total 2051 min (mean 103 min; range 72-150 min). State HV-EM was observed twice as often as was LV-NEM; neither state was identified during 12.4% of the study time. A three-minute moving window was used to calculate r_{max} (mean 0.52; range 0.18-0.77). The relationship between the period of no coincidence and r_{max} and L_{max} was significant (r^2=0.32; p=0.04); however, the low r^2 implies that 68% of the variability is due to other factors. Although this may be the result of inappropriate selection of objective measures, the low r^2 probably reflects the subjective nature of visually assessing FHR-FEM synchronization. Conclusion: Direct computer analysis may provide a more precise measure of fetal state organization.

571 L/S RATIO AS A MARKER OF SEVERE PULMONARY HYPOPLASIA.

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Pulmonary hypoplasia has been associated with a number of pathologic conditions in the fetus. Previous reports have suggested that the amniotic fluid L/S ratio may reflect the degree of pulmonary hypoplasia. 4 cases of fetal pulmonary hypoplasia diagnosed clinically or at autopsy had an L/S ratio obtained prior to delivery. 2 cases involved a diaphragmatic hernia, 1 had a bladder outlet obstruction with severe oligohydramnios, and the remaining was a neuromuscular disorder known to be associated with pulmonary hypoplasia (Pena-Shokeir phenotype). In each case, the L/S ratio was significantly below the gestational mean for a normal pregnancy. In 1 patient with diaphragmatic hernia, L/S was 0.6 and 0.9 at 36 and 37 weeks, betamethasone was given at 36 weeks following the initial amniocentesis and spontaneous delivery ensued at 38 weeks with neonatal death from pulmonary hypoplasia. Thus, an abnormally low L/S ratio may be a marker for a lethal form of pulmonary hypoplasia when associated with fetal conditions in which pulmonary hypoplasia is known to occur.

572 **THE EFFECTS OF VIBROACOUSTIC STIMULATION (VAS) ON BIOPHYSICAL PROFILE TESTING (BPP).** Thomas N. Balaskas, M.D., Joseph A. Spinnato, M.D., University of Louisville School of Medicine, Department of Obstetrics and Gynecology, Louisville, Kentucky.

Vibroacoustic stimulation (VAS) of the fetus has been shown to reduce the number of falsely non-reactive nonstress tests while shortening testing time. The objective of this study was to investigate whether VAS results in improvement of abnormal BPP scores and reduces the latency period for appearance of scoring parameters. ***Methods:*** One hundred four (104) high-risk obstetrical patients between 28-42 weeks gestation with BPP scores ≤ 6 were randomized to VAS (n=51) versus no VAS (n=53). Ultrasound evaluation was continued for an additional 15 minute time period to assess for improvement of score and the time of appearance of scoring parameters. ***Results:*** Of 51 patients who received VAS, 26 (50.1%) showed improvement of BPP score while 21 (39.6%) of the 53 patients randomized to no VAS showed improvement in score (p=NS). Time to appearance of BPP parameters (minutes ± SD) in both VAS and no VAS groups revealed:

	VAS	NO VAS	P
Total Score	(n=30)	(n=25)	
	5.66±5.08	10.8±4.0	<.05
Breathing	(n=9)	(n=12)	
	8.11±6.6	11.6±4.4	NS
Tone	(n=11)	(n=9)	
	6.18±5.1	10±3.5	NS
Gross body	(n=10)	(n=4)	
Movement	4.4±3.5	10±4.1	<.05

Conclusions: Although not statistically significant, VAS applied after BPP testing in patients with scores of ≤6 appears to result in improvement of scores when compared to no VAS. VAS significantly reduced the latency period for appearance of scoring parameters compared to no VAS. These data suggest that VAS may potentially be utilized to shorten testing times and to reduce equivocal or falsely abnormal results.

574 UMBILICAL ARTERY DOPPLER VELOCIMETRY DOES NOT IDENTIFY PERINATAL RISK IN CASES OF OLIGOHYDRAMNIOS. J.R. Wax,* L. Paine,* N.A. Callan, C. Gegor,* T.R.B. Johnson, Dept. Gyn/Ob, The Johns Hopkins Hospital, Baltimore, MD.

Background: Since oligohydramnios and abnormal umbilical artery doppler systolic/diastolic (S/D) ratios are felt to reflect uteroplacental insufficiency, we sought a correlation between these two parameters. Since the minority of patients with oligohydramnios suffer perinatal morbidity, we compared the S/D ratio to the non-stress test (NST) as a means of stratifying perinatal risk in cases of third trimester oligohydramnios. **Methods:** One hundred eighty three structurally normal high risk fetuses underwent simultaneous NST, S/D ratio, and amniotic fluid index (AFI) within seven days of delivery. Data collected on each newborn were: birth weight, ponderal index, hematocrit, 1- and 5- Apgar scores, arterial cord pH and base excess, need for resuscitation or intensive care admission, delivery for distress, and meconium staining. **Results:** No significant correlation was found between the S/D ratio and AFI. In 64 patients with oligohydramnios, the sensitivity of the NST was significantly greater than that of the S/D ratio for detecting risk for low birth weight ($p<.05$), abnormal hematocrit ($p<.05$), arterial cord pH <7.20 ($p<.05$), delivery for fetal distress ($p<.0001$), and ponderal index ≤10% for gestational age ($p<.02$). In 119 patients with normal AFI, the NST was significantly more sensitive than the S/D ratio in detecting risk for abnormal hematocrit ($p<.05$), arterial cord pH <7.20 ($p<.05$), ponderal index ≤10% for gestational age ($p<.05$), and meconium staining ($p<.02$). **Conclusions:** The S/D ratio does not correlate with the AFI in a group of high risk pregnancies. The NST is superior to the S/D ratio in identifying risk for perinatal morbidity by a variety of outcomes in our high risk population.

576 USE OF OXYTOCIN IN PATIENTS WITH PREVIOUS CESAREAN SECTION. B.Gross, A.Fleischer, Dept. Ob/Gyn Albert Einstein College of Medicine, Bronx, N.Y.

This is a retrospective study concerning the efficacy of oxytocin in patients with a previous cesarean section. During the period evaluated, 362 patients with prior cesarean sections were identified 248 of which had a trial of labor. The remaining 114 patients had an elective repeat cesarean section. Oxytocin was used for the usual obstetric indications in 70 patients (28%) irrespective of the reasons for the primary cesarean section. In the absence of oxytocin 124 patients (50%) had a vaginal delivery. An additional 46 women delivered vaginally following oxytocin augmentation, bringing the total rate to 67% ($P<.05$). With this approach, the vaginal delivery rate among patients with CPD as the reason for their primary cesarean section was similar to those with other indications for their primary procedure. Among patients with a primary cesarean section for CPD, 57% delivered a larger baby vaginally. There was no increase in maternal or fetal morbidity in the trial of labor groups compared to elective repeat cesarean sections, nor was there increased morbidity associated with the use of oxytocin among patients delivered by the same route.

577 COMPARISON OF MORBIDITY IN CESAREAN-TUBAL VS. CESAREAN HYSTERECTOMY. Mohammed A Bey, Joseph M Miller, Jr., Joseph G Pastorek,II and Harvey A Gabert, LSU Medical Center, New Orleans, LA.

Cesarean hysterectomy (C-Hyst), often an emergency procedure, is an alternative to cesarean with tubal ligation (C-BTL). Patients undergoing scheduled repeat C-BTL (n=45) were compared to scheduled C-Hyst (n=48). Outcome parameters were compared by t-test, chi square or Fisher exact tests as appropriate.

	C-BTL	C-Hyst	p
Blood Transfusion	8/45	12/48	.397
Post Op Comp	0/45	1/48	1.000
Febrile Morbidity	13/45	9/48	.250
Est Blood Loss (cc)	690±259	1101±450	.0001
Pre-Op Hct (%)	34.7±4.5	35.7±3.4	.2373
Post-Op Hct (%)	31.2±3.6	30.9±5.2	.7495
Δ Hct (%)	3.8±2.8	3.6±2.4	.5983
Op Time (min)	75±21	111±28	.0001
Hosp Stay (d)	4.9±1.4	5.2±2.4	.4566
Patient Age (yr)	26.9±5.1	30.9±5.7	.0007
Gest Age (wk)	38.8±1.7	38.4±1.6	.2471
Parity	3.3±1.5	2.7±1.5	.0577

Operating time and estimated blood loss are increased, but the incidence of blood transfusion and post operative febrile morbidity and other complications were not more common. Elective C-Hyst is a reasonable alternative to C-BTL.

578 SURVEY OF OPERATIVE VAGINAL DELIVERY IN NORTH AMERICA IN 1990. S. Ramin, B. Little*, L. Gilstrap, Dept. Ob/Gyn, Univ. Texas Southwestern Med. Ctr., Dallas, TX

Nearly a decade ago, a survey of obstetric forceps training in North America was published. Since then, the American College of Obstetricians and Gynecologists has published new definitions for forcep deliveries. Our purpose was to survey residency training and current use of obstetric forceps in 1990. Of 294 programs surveyed, 201 (68%) responded, encompassing a minimum of 458,000 deliveries. All but 2 (99%) were familiar with the new definitions which were utilized by 161 (80%) of the programs. The frequency of operative vaginal delivery is summarized below:

	0%	<5%	5-9%	10-15%	16-20%	No Response
Outlet	--	48%	33%	10%	5%	4%
Low	--	60%	30%	4%	1%	4%
	0%	<1%	1-4%	5-8%	>9%	No Response
Mid	14%	59%	25%	0.5%	0%	2%
Vacuum	10%	21%	46%	16%	4%	3%

Attending faculty were the primary instructors in 66% of U.S. and 100% of Canadian programs. Simpson forceps were the most common instrument for outlet (46%) and low (43%) deliveries. Kielland's (27%) and Simpson (24%) were most commonly used for midforcep deliveries. As with the earlier survey, hospitals with high cesarean section rates did not perform significantly fewer midforcep operations. In conclusion, operative vaginal delivery is still commonly taught in residency training programs in North America in 1990. However, the rate of midforcep use apparently has decreased over the past decade (86% vs. 99%). This may reflect the newer, stricter definitions for midforceps utilized by 80% of the respondents.

580 **ACUTE APPENDECTOMY DURING PREGNANCY: A RISK FOR PRETERM LABOR ?** C.Hamel*, L.Leduc. Dept Ob/Gyn, Sainte-Justine Hospital, Montréal, Québec, Canada.

Acute appendicitis is the most common non-obstetrical surgical complication of pregnancy. Generally, the emphasis has been on the diagnostic challenge rather than on the effects of appendectomy on the pregnancy outcome. Therefore, we determined if there is an increased risk of preterm labor after appendectomy. We reviewed the charts of 27 pregnant women admitted to Ste-Justine hospital over a 10-year period. The patient's and gestational ages at admission ranged from 17-37 years (mean: 27 ± 1) and 14-37 weeks (mean: 25 ± 2) respectively. The incidence of preterm labor was higher when appendectomy was performed after 30 weeks of gestation (5/11 vs 0/16, p < 0.01). All delivered within 1 week of surgery and none received prophylactic tocolysis. The mean interval between the admission and the surgery was 15.7 ± 2.0 hrs before 30 weeks and 27.6 ± 7.8 hrs after. The overall rate of misdiagnosed appendicitis was 14.8% (4/27) with a lower rate in the group less than 30 weeks (12% vs 18%). CONCLUSION: 1)Appendectomy appears to increase the risk of preterm labor after 30 weeks of gestation. 2) Prophylactic tocolysis should be considered in these cases.

581 **THE RELATIONSHIP BETWEEN THE BISHOP SCORE AT 41 WEEKS AND THE DURATION OF POSTDATES PREGNANCIES.** KB Porter, WF O'Brien, T Nguyen,* L Johnson,* E Brooks,* J Holbrook.* University of South Florida College of Medicine, Tampa, Florida.

A retrospective chart review of 1,268 women delivering at 41 weeks or later over a 3 year period were evaluated. Of this population, 389 women entered a postdates screening program at 41 weeks. One hundred and eight had assured dates having had either a first or second trimester sonogram. When comparing those women with confirmed versus unconfirmed dating, no differences were found in the mean Bishop score, incidence of nulliparity, or in the rate of induction (30%). In both groups those women with unfavorable Bishop scores delivered at a later gestational age.

	% Delivered		
Bishop Score	41-42 wk.	42-43 wk.	≥43 wk.
0-5	47.40	35.84	16.76
6-8	60.38	33.96	5.66
>8	93.75	6.25	0

In conclusion, cervical condition at 41 weeks regardless of dating accuracy strongly predicts the likelihood of pregnancy lasting beyond 42 weeks.

583 Prenatal Care of the Adolescent. C.J. Sims, H.R. Giles, Dept. of Ob/Gyn, Medical College of Pennsylvania/Allegheny Campus, Pittsburgh, PA.

In an effort to provide optimum care of the pregnant adolescent a review of the needs of the adolescent in our community was undertaken. Educators, county officials, health care providers, adolescents and community leaders were interviewed. The consortium agreed upon the importance of early, consistent, quality prenatal care that enhanced the long term quality of life of the adolescent family. The importance of continuation of her education ranked high on the list of needs. Therefore, an agreement was made between the city school board and a local health care provider for school based prenatal care. A consistent team of providers was established. Members included: 1) Educational director of the school-based clinic, 2) Maternal-Fetal medicine specialist with an interest in adolescent pregnancy, 3) perinatal nurse specialist/case manager, 4) school nurse, 5) nurse's assistant, 6) Adolescent medicine specialist (providing long term care for the adolescent and her infant), and 7) psychiatric social worker. The implementation of this model program was an example of a community's combined efforts enacted to address the alarming concerns of inner city adolescents. After two years of operation, the consortium agrees that this program has a salutary impact. This program is depicted as a successful model of a synergistic health care/educational environment.

584 SHOULDER DYSTOCIA: RELATIONSHIP BETWEEN NEONATAL INJURIES AND ACID BASE STATUS. IA Hoskins, P Ehrlich,[1] SA Ordorica, BK Young, RF Porges,[1] Dept. Ob/Gyn, NYU Med. Ctr., New York, NY.

Morbidity with shoulder dystocia (SD) may be due to delayed delivery and asphyxia. We reviewed 13,440 deliveries from 1/1/81 to 5/1/91 to identify SD and correlate severity of injuries with acid base status. There were 81 cases with complete followup in 67 (83%). Neonates were grouped according to severity of injuries. Group I (n=30, 45%) no injuries; group II (n=26, 39%) moderate injuries eg. bruising, hypotonia; group III (n=11, 16%) severe injuries eg. fractures, palsy. Overall incidence of acidosis (pH < 7.20) was 43%. It was 81% in group II vs. 7% and 55% in groups I and III ($p < 0.05$). Of the 6 acidotic neonates in group III, 4 (67%) had palsy whereas 2 (33%) had isolated fractures, $p < 0.05$. Incidences of low (≤ 3) Apgar scores were 7%, 19% and 18% in the 3 groups (NS). There was no correlation between severity of injury and meconium, seizures, hypotonia or apnea. Excessive, prolonged traction on the infant (causing hypotonia, palsy) was associated with neonatal acidosis whereas fractures appeared to expedite delivery and prevent acidosis.

585 FETAL DETERMINANTS OF ASSISTED VAGINAL DELIVERY. M.C. Williams, W.F. O'Brien. Department of Obstetrics and Gynecology, University of South Florida, Tampa, FL

Prior investigations of successful vaginal delivery have identified significant correlations with such factors as fetal weight and maternal pelvic dimensions, while the association with fetal asymmetry, as assessed by the ponderal index (birthweight/length^3), is unknown. Data were compared between 126 control vaginal deliveries, and successful assisted vaginal deliveries for cephalopelvic disproportion (72) and fetal distress (99). Chi-square goodness of fit comparisons between the control group and population norms found them similar for birthweight, crown-heel length, and ponderal index percent for gestational age, while head circumference percent among controls was slightly smaller than expected (mean 47%, $P<0.05$). The three groups were found similar for maternal height, weight, previous cesarean section, labor induction, meconium, gestational age, and birthweight. Ponderal index percent was significantly associated with assisted delivery ($P<0.004$). Logistic regression for need for assisted vaginal delivery found previous successful vaginal delivery ($R=0.23$, $P<0.0001$), ponderal index % ($R=0.16$, $P<0.001$), and head circumference % ($R=0.13$, $P<0.004$) formed a model which correctly assigned 68% of cases with a non-significant model chi-square goodness of fit ($P<0.42$). Maternal height, weight, and history of previous cesarean were not significantly associated. Ponderal index % is correlated with assisted delivery.

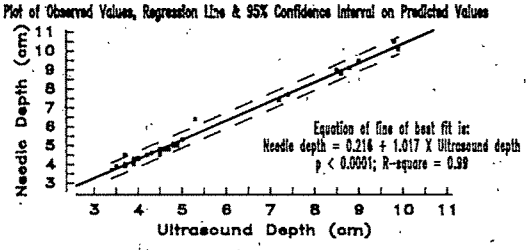
	NSVD	CPD	DISTRESS	SIG.
BIRTH WEIGHT %	48	51	42	NS
HEAD CIRCUMF %	47	58	51	<0.04
PONDERAL IDX %	49	40	37	<0.004
UMB ARTERY pH	7.28	7.29	7.22	<0.0001
BASE DEFICIT	3.6	4.2	5.4	<0.0001

587 INDIRECT SONOGRAPHIC GUIDANCE FOR EPIDURAL ANESTHESIA IN OBESE PREGNANT WOMEN DELIVERED BY CESAREAN SECTION

D. Wallace, M.D.[1], L. Gilstrap, M.D., J. Currie, M.D.[1], and R. Santos, M.D.[1]
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Obesity and edema which obscured lumbar landmarks resulted in technical difficulties and prolonged our attempts to achieve epidural anesthesia. However, indirect sonographic guidance with use of either the Toshiba SAL-32B or RT 3000 GE machine with 5mHz transducer (visual field width 5.6 or 8cm) has allowed us to identify the midline by saggital scan of the easily imaged laminae of lumbar vertebrae 2,3,4; to predict needle depth (ND) from ultrasound depth (UD, skin-to-lamina distance); and knowing UD, the midline, and transducer center site marked where positioned over the 2nd or 3rd interspace before its removal, percutaneously advance a 9.5 or 11.4cm Tuohy needle perpendicularly from the site until epidural puncture (EP). We prospectively studied 36 obese women scheduled for elective repeat C/S. ND was measured by marker and measure, and epidural anesthesia was successfully administered to all patients. Simple linear regression analysis was performed, with strong positive results (See figure).

Plot of Observed Values, Regression Line & 95% Confidence Interval on Predicted Values



Indirect and continuous sonographic guidance studies, designed to determine if sonography reduces complications, are currently being planned.

588 ADENOSINE INDUCES TACHYCARDIA AND LOWERS BLOOD PRESSURE IN THE PREGNANT EWE. Brian A. Mason, MD[1], Brian J. Koos, MD, DPhil. Dept. of Obstetrics and Gynecology, Nicholas S. Assali Perinatal Research Laboratory, UCLA School of Medicine, Los Angeles, CA 90024

Adenosine has recently been approved by the FDA for termination of paroxysmal supraventricular tachycardia (PSVT). Due to its very short half life, this purine nucleoside may be particularly useful in pregnant women with PSVT. Because adenosine can cause tachycardia and hypotension, the relative safety of this agent must be established before it can be recommended for general use in pregnancy. We therefore investigated the effects of graded intravenous infusions of adenosine in three chronically catheterized gravid ewes (>0.8 term). Infusions were increased in a stepwise manner from 25 to 400 μg/kg/min by doubling the infusion rate at five minute intervals. The same procedure was performed in reverse, beginning at 400 and decreasing to 25 μg/kg/min with results being similar in both cases. No significant changes occurred in mean pH, PCO_2, or PO_2. Mean heart rate increased from 110 ± 3.7 to 173 ± 4.3 BPM ($P<0.001$) with statistically significant response ($P<0.05$) noted at infusion rates as low as 50 μg/kg/min. MAP decreased from 85.5 ± 3.4 to 79.4 ± 4.5 mmHg ($P<0.01$). Conclusion: While the effects on MAP and HR are statistically significant, they probably pose no clinical risk to gravidas because the effects are within a range which is tolerated physiologically and are transient. Possible fetal effects of maternal adenosine infusions require further investigation. Supported by HD-18478.

589 FETAL ASCITES FLUID - A NEW SOURCE OF CELLS FOR CHROMOSOMAL ANALYSIS. J.R. Wax,* K.J. Blakemore, G. Stetten,* Dept. of Gyn/Ob, The Johns Hopkins Univ. Sch. of Med. Baltimore, MD.

Background: The optimal source of cells for chromosomal analysis of the sonographically anomalous fetus is influenced by the accessibility of the cells, procedure-related risks, and the speed with which results may be obtained. We have rapidly and reliably performed cytogenetic analysis using cells cultured from fetal ascites. **Methods:** Fetal ascites was obtained during therapeutic paracentesis from two patients at 33 weeks and 19 weeks gestation. Following a differential cell count, ascitic fluid was set up at 10^6 cells/ml of media, stimulated with phytohemagglutinin (PHA), synchronized, and harvested at 96 hours according to a standard lymphocyte protocol. **Results:** In the first patient, the cultured ascites cell karyotype confirmed the diagnosis from fetal blood lymphocytes and amniocytes as 45,X. In the second patient, the ascites cell karyotype confirmed the amniocyte diagnosis of 47,XY,+13. **Conclusion:** Fetal ascites is an easily accessible source of cells for culture and rapid karyotype. The fluid is readily visible sonographically and easily removed without undue fetal risk by ultrasound-guided needle aspiration. More rapid cytogenetic results should be possible as with peripheral blood lymphocytes using shorter culture times.

590 PRENATAL DIAGNOSIS OF BIRTH DEFECTS. A REVIEW OF 67,349 DELIVERIES. D. Ogunyemi[X], P. Brown[X], R. Willis-Hassan[X], M. Hernandez, T. Fukushima. King-Drew Medical Center, Los Angeles, CA.

This study was undertaken to examine the efficacy of current practices of prenatal care in detecting birth defects. Methods: All deliveries from 1982 through 1989 were evaluated; chi-square test was used for statistical analysis. Results: A total of 385 cases of birth defects were identified (5.7/1000). Birth defects were considered amenable to prenatal diagnosis in 250 (66%) cases and not detectable by current technology in 135 (34%). However, the actual number of cases detected by routine prenatal care was only 67 (27%). In 183 potentially detectable cases diagnoses were not made for the following reasons: No PNC (26%); had PNC but was not tested (50%); tested but defect was missed (4%); too young for amniocentesis (30%).

BIRTH DEFECTS	CNS	M-S§	CARDIAC	CHROMOS.	PN Deaths
Detected (67)	45(41%)	5(31%)	2(10%)	11(16%)	39(59%)
Not detec.(183)	64(59%)	11(69%)	19(90%)	63(84%)	45(25%)

(All differences p<.0001) § M-S = Musculo-Skeletal

Conclusions: In this population, the majority of birth defects was not detected prenatally, although only 4% were missed when appropriate diagnostic techniques had been used. To diagnose all potentially detectable cases, the following tests would have been required: Ultrasound (60%); amniocentesis (30%); and fetal echocardiography (10%). A more liberal use of these procedures, including amniocentesis for younger women (<35 y/o), may enhance the rate of detection of birth defects.

591 THE PREVALENCE AND CLINICAL SIGNIFICANCE OF THE ISOLATION OF UREAPLASMA UREALYTICUM IN MIDTRIMESTER AMNIOCENTESIS. M. Mazor, S. Horowitz,[X] R. Romero, C. Walter,[X] M. Glezerman,[X] Depts. of Ob/Gyn, Soroka Med. Center, Ben Gurion Univ., Israel and Yale Univ. School of Medicine, New Haven, CT

The causes of pregnancy loss following midtrimester amniocentesis are unknown. Pre-existing subclinical microbial invasion of the amniotic cavity may be a predisposing factor for membrane rupture and chorioamnionitis following the procedure. The purpose of this study was to determine the frequency and clinical consequences of microbial invasion of the amniotic cavity in women undergoing midtrimester amniocentesis for genetic indications. This study focused on Mycoplasmas (Ureaplasma urealyticum and Mycoplasma hominis) because these are the microorganisms most frequently isolated in cases of microbial invasion of the amniotic cavity. Materials and Methods: Amniocentesis for genetic indications was performed in 193 consecutive patients. Amniotic fluid was cultured for Mycoplasma hominis and Ureaplasma urealyticum using methodology previously described. Cervical cultures were obtained following amniocentesis. Follow-up was available in all patients. Results: The prevalence of positive amniotic fluid cultures was 2.5% (5/193). Ureaplasma urealyticum was the only isolate from amniotic fluid. The rate of spontaneous preterm delivery was higher in patients with a positive amniotic fluid culture than in patients with a negative culture (42.8% [3/7] vs. 5.3% [10/186]; p <0.05). One patient with a positive culture ruptured her membranes one hour after the amniocentesis. (All patients with a positive amniotic fluid culture had Ureaplasma urealyticum isolated from the lower genital tract.) Conclusions: 1) Microbial invasion of the amniotic cavity with Ureaplasma urealyticum was detected in 2.5% of women undergoing midtrimester amniocentesis. 2) Pre-existing microbial invasion of the amniotic cavity can be responsible for a fraction of post-amniocentesis pregnancy loss. 3) Colonization of the amniotic cavity with Ureaplasma urealyticum is a risk factor for preterm delivery. 4) Routine culturing of amniotic fluid for Mycoplasmas must be considered at the time of midtrimester amniocentesis.

592 **FOUR YEAR REVIEW OF PRENATAL DIAGNOSIS BY PUBS.** R.D. Wilson, D.F. Farquharson[x], D. Shaw[x], B.K. Wittmann[x]. Dept. Ob/Gyn, Univ. British Columbia, Grace Hospital, Vancouver, B.C. Canada.

Fetal evaluation by percutaneous umbilical blood sampling has allowed a more rapid diagnosis of fetal conditions. We present our four year experience with 214 PUBS procedures. Indications included fetal anomalies requiring chromosome diagnosis in 135 (63%), evaluation of fetal platelets 49 (23%), maternal Rh disease 24 (11%), and miscellaneous indications in 6 (3%). For the chromosome indication 122 were for fetal karyotypes and 13 for fragile X evaluation. The number of failed procedures was 15 (7%). The number of post-procedural deaths was 4 (1.9%), and neonatal deaths 2 (0.9%) with an overall loss rate of 2.8%. The most common malformations necessitating chromosome diagnosis were intrauterine growth retardation (21), fetal hydrops (17), central nervous system abnormalities (15), and multiple congenital anomalies (14). Fetal blood sampling to evaluate fetal mosaicism was used in 7 cases where CVS or amniocentesis had indicated possible mosacism. Gestational ages at the time of the procedure was equally distributed between 5 gestational age groups - less than 20 weeks, 20-25 weeks, 25-30 weeks, 30-35 weeks, and greater than 35 weeks.

593 TRANSABDOMINAL-GUIDED TRANSVAGINAL CHORIONIC VILLUS SAMPLING. LP Shulman, JL Simpson, OP Phillips[X], RE Felker[X], DS Emerson[X], S Elias. University of Tennessee, Memphis.

Transvaginal chorionic villus sampling (CVS) using *endovaginal* ultrasound guidance has been previously described to obtain chorionic villi from patients who desire CVS but have placentas inaccessible to either transabdominal (TA) or transcervical (TC) approaches. We describe here use of *transabdominal*-guided transvaginal CVS in 9 women undergoing CVS. An ultrasound examination is first performed to evaluate placental location and presence of intervening structures (e.g., bowel, blood vessels). TA ultrasonography is then performed to visualize the needle used for anesthetic infiltration. If the needle is clearly visualized, we proceed with TA ultrasound-guided transvaginal CVS. A 35 cm, 18-gauge aspiration needle (Cook Urological Inc) is inserted through the posterior wall of the vagina and uterus into the placenta. A 20 cc syringe containing 4 cc transport medium is attached to the needle hub. Chorionic villi are obtained by 10-15 aspirations of the syringe plunger to 20 cc negative pressure; upon completion, the needle is removed under continuous negative pressure. Patients are monitored for 15 minutes for any untoward effects (e.g., hemorrhage) prior to discharge. **RESULTS:** We have performed this CVS technique to obtain chorionic villi from 9 patients (mean gestational age: 11.2 weeks). Adequate samples were obtained with only a single needle passage in all 9 women. Direct and culture cytogenetic results were obtained from all 9 specimens and all analyses revealed normal complements. There were no immediate or long-term complications (i.e., maternal infection, hemorrhage, pregnancy loss). Six patients have been delivered at term of healthy infants, whereas 3 pregnancies are continuing uneventfully. **CONCLUSION:** Transabdominal-guided transvaginal CVS is a useful method for obtaining chorionic villi in a select group of patients, specifically those women with placentas located posteriorly within a retroverted, retroflexed uterus. However, CVS by either transcervical or transabdominal aspiration is preferable until the safety and accuracy of the transvaginal approach is known.

594 **EFFECTS OF PRENATAL EXPOSURE TO METHANOL AND T-BUTANOL IN LONG EVANS RATS.** E.L. Abel[X] and P.J. Bilitzke.[X] Department of Obstetrics/Gynecology and Fetal Alcohol Research Center, Wayne State University School of Medicine, Detroit, MI.

Pregnant rats consumed liquid diets containing methanol (1.6%, 0.9%, 0.6% v/v) or t-butanol (10.9%, 1.3%, 0.65% v/v) beginning on gestation day 8 until parturition. Each group had its own pair-fed controls. After parturition mothers were put on lab chow ad lib. Methanol did not affect fecundity but reduced maternal weight gain, decreased litter sizes (from 12 to 5 pups per litter), increased perinatal mortality (from 4% to 25%) and postnatal mortality (from 0% for controls to 100% for offspring in the highest dose group), and decreased weights at weaning for survivors in the other methanol groups. Since methanol treated animals did not differ from pair fed controls in weight gain, these effects could not be due to decreased maternal weight gain. T-butanol reduced maternal weight gain, litter sizes (from 11 to 3 pups per litter), birth weights, and weights at weaning and increased perinatal mortality (from 2% to 14%) and postnatal mortality (from 6% to 100%). These results indicate that prenatal exposure to methanol and t-butanol can result in very high postnatal mortality rates. These rates are much higher than we have previously seen in connection with prenatal alcohol exposure. Supported in part by grant P50 AA07606 from NIAAA.

595 AMNIOTIC FLUID VOLUME AND PRENATAL GROWTH DISTURBANCES IN FETUSES WITH MONOSOMY X OR XO MOSAICISM. J Cullen[X], J Abramowicz, L Metlay[X], Depts. Ob/Gyn & Pathology, Univ. Rochester Sch. of Med., Rochester, NY

Sonographic and/or autopsy information on 13 fetuses with Monosomy X and 6 fetuses with Turner mosaicism (XO/XX or XO/XY) was retrospectively reviewed. Fetal growth was assessed by sonographic parameters and/or autopsy measurements. Amniotic fluid volume (AFV) was recorded from ultrasound (U/S) reports. Pathologic data regarding structural anomalies was recorded as well. Altered fetal growth was demonstrated in 8/10 fetuses with Monosomy X and 2/5 fetuses with Turner mosaicism. In both groups, normal AFV was seen prior to 16 weeks gestation (GA). U/S data after 16 weeks was available for 11 fetuses with Monosomy X, and severe oligohydramnios was demonstrated in 10 of these. U/S data was available for 5 fetuses with XO mosaicism. 4/5 pregnancies underwent termination prior to 20 weeks GA. Serial U/S data was available for the fifth pregnancy; normal AFV was seen until 25 weeks GA, at which time oligohydramnios was identified. No structural anomalies were found in the mosaic population. Frequently noted structural anomalies in fetuses with Monosomy X included cystic hygroma, hydrops fetalis, hypoplastic aortic arch, and single umbilical artery. No renal anomalies were identified. Placental findings, though suggestive, were nondiagnostic. Conclusion: Fetal growth alterations and severe oligohydramnios frequently occur in association with Monosomy X. The etiology of these findings remains unclear.

597 RACIAL DIFFERENCES IN BIRTH DEFECTS IN A LOW INCOME MINORITY POPULATION. A REVIEW OF 67,349 DELIVERIES. D.Ogunyemi,* P.Brown,* R.Willis-Hassan, T.Fukushima, King/Drew Medical Center, Los Angeles, CA.

Previous studies have suggested that the high perinatal mortality rate in low income Black women may be due in part to congenital defects. The purpose of this study was to determine if there were differences in the prevalence and types of birth defects in low income Black and Hispanic women and assess the associated prematurity and mortality rates. All deliveries from 1982 to 1990 were included; the data were evaluated with Chi-square test. Results:

	Blacks	Hispanics	
No. Cong. Anomalies	81	295	
Rate/1000	10.7	7.6	p<0.0001
CNS defects	28%	31%	p<0.05
Facial-oral defects	9%	16%	p<0.05
Cardiac defects	14%	6%	p<0.05
Genital defects	7%	2%	p<0.05
Chromosomal defects	14%	21%	p<0.05
LBW	53%	30%	p<0.0001
Preterm births	42%	20%	p<0.001
Postdate pregnancy	10%	21%	p<0.001
Perinatal mortality rate	221	237	NS

Conclusion: There was a significantly increased incidence of birth defects in Blacks vs Hispanics, with a different pattern distribution (cardiac vs chromosomal). Birth defects in Blacks were more likely to be accompanied by low birth weight and prematurity. The similar mortality rate in both groups suggests that defects in Blacks are more compatible with survival.

600. **PURE PLACENTAL TRISOMY 16 ASSOCIATED WITH A 46,XY INFANT AND SEVERE PREECLAMPSIA: A CASE REPORT.** K. K. Vernof [x], J. A. Ney, G. W. Dewald [x]. Departments of Ob/Gyn and Cytogenetics, Mayo Clinic. Rochester, Minnesota.

We report the clinical presentation, placental pathology, and cytogenetic studies of a rare case of trisomy 16 placenta associated with a 46,XY infant. The patient, a 32-year-old gravida 2, para 1, was referred at 32 weeks gestation for evaluation of hypertension, proteinuria, and hyperreflexia. Shortly after admission, an emergent cesarean section was performed for prolonged fetal bradycardia. A 1120 gram male was delivered, and although he weighed less than the 10th percentile for age, the infant appeared normal. The placenta was grossly abnormal with multiple, diffusely located hydropic villi. Cytogenetic studies revealed 29 of 29 metaphases from the placental biopsy with trisomy 16 and 50 of 50 cells from cord blood with 46,XY. We hypothesize nondisjunction very early in development as an explanation for a trisomic placenta and karyotypically normal neonatal lymphocytes. Aneuploid placentation is a possible etiology for the placental insufficiency, severe preeclampsia, and intrauterine growth retardation observed in this case.

601 **NEW EXPERIENCES WITH THE PRENATAL DIAGNOSIS AND THERAPY OF FETAL PARVOVIRUS B19 INFECTION.** W. Holzgreve, T. Schwarz, M. Evans, B. Holzgreve, Dept. Ob/Gyn, Univ. of Münster, Dept. of Virology, Univ. of München, FRG; Wayne State Univ./Hutzel Hospital, Detroit, MI

Maternal parvovirus B19 infection in pregnancy causes hydrops fetalis leading to fetal death in up to 10% of the cases. After anemia due to aplastic crisis is confirmed by cordocentesis, intrauterine transfusions are now routinely performed by our group. In our series in one case, however, we encountered intrauterine fetal demise despite successful treatment of hydrops. This may be due to a direct cardiac effect of B19 in utero. We also found that the sensitivity of dot blot hybridization for the detection of prenatal B19 infection is only sufficient in fetal blood with a high level of viremia. We further encountered a case of severe hydrops, eight weeks after onset of maternal exanthema, in which at the time of aplastic crisis in the fetus both maternal and fetal blood were negative for anti-B19 IgM. In this case fetal infection could ultimately be confirmed by PCR in amniotic fluid, ascites and fetal blood. The dramatic life-threatening state of the fetus required intrauterine transfusions already before laboratory confirmation of fetal B19 infection was available.

602 **THE IMPACT OF PREVIOUS LOW BIRTHWEIGHT ON FETAL GROWTH IN THE CURRENT PREGNANCY.**

RL Goldenberg, HJ Hoffman,* SP Cliver,* GR Cutter,* RL Copper.*
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A history of low birthweight (HLBW) in a previous pregnancy is associated with low birthweight in the next pregnancy. However, the effect of HLBW on the gestational age at delivery and various newborn anthropometric measurements is less clear. In 1545 pregnancies, 38% with a HLBW (defined as a birth <2750 g) and using multiple regression techniques adjusting for maternal race, age, height, weight, weight gain, hypertension, smoking, alcohol and drug use, the effect of HLBW was evaluated. Women with HLBW had a 23.4% rate of preterm delivery compared to 9.5% in women without this history. ($p < .001$) The mean gestational age for HLBW was 37.7 ± 3.2 weeks vs 38.7 ± 2.9 wks in women without HLBW. ($p < .001$) The mean birthweight for HLBW women was 2913 ± 669 g vs. 3219 ± 651 g for non-HLBW women ($p < .001$), a difference of 306 g. Of this a difference of 128 g was associated with preterm birth, with 178 g associated with differences in size in term births. Smoking was associated with a decreased weight of 149 g and black race a decrease of 148 g, with maternal hypertension, drug use, height, weight, and weight gain all having a significant impact on weight. However, even adjusting for these factors, HLBW was associated with a 107 g reduction in birthweight. While all anthropometric measurements were less in infants with maternal HLBW, the differences in length measurements and all skinfolds were not significant. However, the head, chest, abdomen, arm and thigh circumferences as well as the ponderal index were all significantly smaller. These results suggest that the pattern of growth restriction associated with HLBW resembles the condition described as asymmetric growth retardation. However, because the skinfold measurements were not significantly reduced in relationship to HLBW, the pattern of growth restriction associated with HLBW is not typical of asymmetric growth retardation.

604 **ONE AND 5-MINUTE NEONATAL OXYGEN SATURATION: CLINICAL CORRELATIONS:** D.Faustin, B. Podbielski,[x] D.Gotlin,[x] Y. Fuchs,[x] S. Mccalla,[x] K. Violaris,[x] H. Minkoff, Dept OB/GYN, SUNY/Health Science Center, Brooklyn, NY. **Introduction:** Successful adaptation of newborns to extrauterine life depends on their ability to carry oxygen in sufficient amount to vital organs. Little is known however, about how useful noninasive O_2 saturation monitoring can be in the delivery room during early adaption. **Materials and Methods:** We studied the umbilical cord oxygen saturation (O_2 sat) at birth and compared it with the 1 and 5-minute transcutaneous neonatal O_2 Sat in two groups of term infants: Group A comprised 14 neonates with no abnormal fetal heart rate patterns during labor and no difficulties at delivery; Group B, 10 newborns with such complications. The prenatal course was normal in both groups and delivery occurred between 38 and 42 weeks. Mean cord pH and O_2 Sat at birth, O_2 Sat at 1 and 5 minutes (pulse oximeter SENTINEL 2,000) and change in O_2 Sat from 1 to 5 minutes were compared between groups A and B. Student t-test and X^2 were used for statistical analysis with $p<.05$ considered significant. **Results:**

	Mean Birth Cord pH	O_2 Saturation birth	1 min	5 min	Decreased in O_2 Sat from 1-5 min (by at least 5%)
Group A n=14	7.31±.05	38.8±22	66.8±17	89.4±10	0
Group B n=10	7.25±.05	29.5± 31	85±11.5	81.0±12.7	7
Signifance	p<.02	NS	p<.01	NS	p<.005

There was no statistical difference in Apgar scores at 1 and 5 minutes between the two groups. **Conclusions:** There was a significantly lower mean cord pH at birth between groups A and B. In this preliminary study a decrease in O_2 saturation from 1 to 5 minutes by at least 5% was significantly associated with fetal monitoring signs of fetal distress. This may represent a compensatory mechanism to maintain adequate neonatal tissue oxygenation in the midly acidotic neonate.

606 FDCREP- A DATABASE DESIGNED FOR ANTENATAL DIAGNOSTIC SERVICES. D. Lagrew, H. Doan[X], R. Steiger, Dept Ob/Gyn, Saddleback Memorial Medical Center, Laguna Hills, CA., and Univ. of California, Irvine, Orange, CA.

Antenatal diagnostic centers follow patients with a range of prenatal diagnostic services which including ultrasound, genetic sampling and fetal well being studies. A comprehensive database was developed to collect data, generate reports and analyze results of diagnostic services. The application was developed in RBASE, a PC-based database language. FDCREP is operating efficiently on a network system with 9 work stations. Data entry has been expedited by numeric coding. Redundant entries are minimized so that the system is time efficient. After entering data on 2932 patients, 4155 ultrasounds, 972 amniocenteses and 3479 antepartum tests, the database occupies 6.4 Meg of disk space. Data entry forms for patient demographics, past medical history, ultrasound, amniocentesis, and fetal testing have been developed. Ultrasound calculations are made at the time of entry. Reports are generated for ultrasounds (gynecologic, 1st trimester, obstetrical), fetal well being (CST,NST,BPP,AFV), and amniocentesis (letter, tabular). In addition chronologic summary reports of fetal well being results can be produced. Summary reports on numbers of tests, types of tests and referral usage are generated. The system allows for quality assurance analysis and research statistics. In summary a comprehensive database for antenatal services has been developed which requires a minimal amount of memory for archiving.

607 LDLOG - A DATABASE DESIGNED FOR SUMMARIZING AND MAINTAINING LABOR AND DELIVERY STATISTICS. D. Lagrew, H. Doan[X], R. Steiger, Dept Ob/Gyn, Saddleback Memorial Medical Center, Laguna Hills, CA, and Univ. of California, Irvine, Orange, CA.

Rapid and accurate statistics of labor and delivery information are required for hospital, local and state agencies. Such analysis allows for utilization analysis and quality improvement. In order to expecite entry and analysis a computerized labor and delivery database was developed. Written in RBASE, a PC-Based database program, the forms were developed with maternal and neonatal information. Numeric coding has allowed efficient storage with nearly 6000 deliveries being stored in 3.7 Meg of hard disk space. The efficient use of memory utilized by this program allows for the archiving of many years of data on one hard disk. A monthly summary including labor and delivery statistics, log of deliveries, and physician statistics are generated by the application. A breakdown of cesarean rates, indications and VBAC attempts are generated. Individual cesarean section reports analyze each physician's rate and risk factors. Summary logs by physician can be generated. The results are utilized for quality improvement and utilization review. The timely feedback has allowed physicians to evaluate their own practice patterns.

608 A MULTI_USER PC AND MACINTOSH PERINATAL ULTRASOUND DATABASE FOR CLINICAL AND RESEARCH USE.

Beattie RB, McLaughlin P[X], Dept. Obstetrics/ Gynaecology, Queens University Belfast, Northern Ireland.

A customised ultrasound database based on commercially available software (Omnis 5- Blythe Software) has been developed to meet the service and research needs of a regional referral unit with file sharing on a mixed PC and Apple Macintosh network. The user-interface is predominantly icon driven and affords easy and rapid entry of data with minimal typing and "point and click" selection of most options. The hierarchical relational database design permits unlimited entry of multiple pregnancies and ultrasound examinations with easy searching using standard and user-defined searches to identify mothers, fetuses or ultrasound examinations which meet the search criteria. Biometric and menstrual data are used to calculate gestational age and estimated fetal weight based on published formulae and to generate customised pregnancy specific growth curves based on an individual mother's demographic data and past obstetric history. Data export to statistical and graphical programmes is easily accomplished whilst textual reports including management reccomendations are generated for clinical use and output to file, screen or printer as required. Doppler studies and ultrasound guided procedures such as CVS, Amniocentesis and Cordocentesis are catered for and a simple Perinatal Outcome module is included to allow the system to be used as a stand alone Perinatal database.

609 PRETERM BIRTH PREVENTION IN A LARGE MEDICAID POPULATION. R.C. Floyd,[X] R.W. Martin, K.S. Gookin,[X] W.E. Roberts, B.N. McLaughlin,[X] J.C. Morrison, Dept. Ob/Gyn, Univ. Mississippi Med. Ctr., Jackson, MS

Objective: Determine the results of a comprehensive program of premature birth prevention in Medicaid women. Population: Over a 70-month period, 4008 pregnant Medicaid patients (Group I) from 47 states received patient education, frequent cervical examinations, daily nurse contact and home uterine monitoring (Tokos Medical Corp., Santa Ana, CA). These were compared to a matched group (II) of women from one state (MS) who received high-risk standard care (N=91). Those delivered for medical indications or because of patient/physician non-compliance were not included (N=996, 20 respectively). Main Outcomes Measured: In this retrospective study, the gestational age at diagnosis of preterm labor (PTL) and delivery, interval between tocolysis and birth in those with PTL, incidence of PTL, and number delivering delivering preterm were recorded. Results: In the 4008 patients there were 8702 risk factors, 2280 (26%) were for PTL during the current pregnancy. Preterm delivery or PTL in a prior pregnancy and multifetal gestations accounted for 43%. Multiple risk factors comprise the other 31% of monitored subjects. The risk factor percentage was similar in Group II.

Group	Number	PTL	GA at Diagnosis	PTD	GA at Delivery
I	3012	65%	30.0	21%	265 ± 14 d
II	71	61%	29.3	46%	238 ± 12 d

The percentage of women with PTL in each group was similar as was the gestational age at diagnosis of PTL. The gestational age at delivery ($P < .001$) was lower and the number delivering < 37 weeks ($P < .001$) was higher in the group receiving standard care. Conclusions: In a large Medicaid population, a comprehensive program of intensive perinatal nurse assessment, ambulatory uterine monitoring, and aggressive provider care rendered better results concerning preterm delivery percentage than did a program of high-risk care alone.

611 FETAL RENAL BIOPSY: TECHNIQUE DEVELOPMENT. W.A. Campbell, H.T.Yamase,* C.M.Salafia,* A.M.Vintzileos, J.F.Rodis. University of Connecticut Health Center, Farmington, Ct.

Intrauterine vesico-amniotic shunting for fetal obstructive uropathy, can prevent irreversible renal damage which causes renal dysplasia. Technical success has been achieved. However, current case selection criteria using fetal urine electrolyte profile and ultrasound appearance, have shortcomings. We hypothesize that a fetal renal biopsy might detect dysplasia and improve case selection. This study reports initial work towards defining an adequate needle gauge for fetal renal biopsy. **Methods:** Fresh autopsy kidney specimens from 16 to 40 weeks were used. Needle biopsy aspiration was obtained using 20, 18, 16, &14 gauge needles. Biopsies underwent fixation and preparation for histologic examination. Biopsy specimens were evaluated for the recognizable presence of cortex and medullary structures. Biopsy grading: completely adequate (CA)-both cortex & medulla present, partially adequate (PA)-only cortex or medulla present, not adequate (NA)-only part of cortex or medulla present, & no sample (NS)-tissue did not survive processing. **Results:** 75 biopsies were taken from 18 kidneys: 20g (17), 18g (20), 16g (19), 14g (19). Of these, 39/75 (52%) were CA, 24/75 (32%) were PA, 2/75 (3%) NA, and 10/75 (13%) NS. Needle gauge evaluation Table 1.

	20 gauge	18 gauge	16 gauge	14 gauge
CA	35.0 %	25.0 %	69.0 %	79.0 %
PA	47.0 %	35.0 %	26.0 %	21.0 %
NA	6.0 %	5.0 %	0.0 %	0.0 %
NS	12.0 %	35.0 %	5.0 %	0.0 %

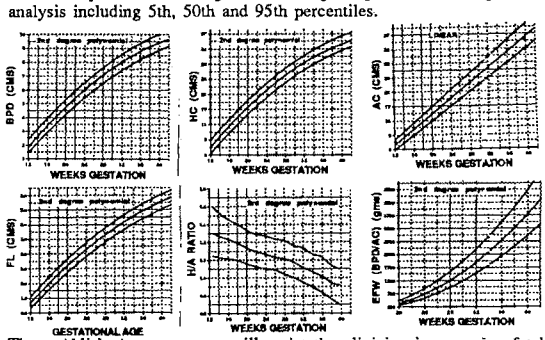
Summary: Adequate biopsies (CA,PA) were obtained in 84% of the specimens; 62% were CA. The 16 & 14 gauge needles give the best results. However, the aspiration technique fragments the specimen; small fragments are lost during processing and account for NA and NS results. A cutting biopsy may avoid this and provide a better idea of a needle gauge adequate for evaluation.

612 **CHEEK TO CHEEK DIAMETER IN SONOGRAPHIC ASSESSMENT OF ABNORMAL FETAL GROWTH**
Jacques S. Abramowicz, M.D., David M. Sherer, M.D., James R. Woods, Jr., M.D., University of Rochester, Rochester, NY

The fetal cheek to cheek diameter (CCD) and its growth have previously been described in 200 normal control pregnancies (SPO 1991, Abstract #33). The CCD was found to be linearly correlated to gestational age (GA) from 20 to 41 weeks. The CCD/BPD ratio was almost GA independent (ranging from 0.6 ± 0.08 to 0.7 ± 0.08). In the current study, we evaluated the CCD and CCD/BPD ratios in 110 fetuses with the following growth disturbances: IUGR, EFW <10th percentile (n=16) and macrosomia, EFW >90th percentile with (n=30) or without (n=64) maternal diabetes mellitus. Both CCD and CCD/BPD ratios were significantly smaller in IUGR as compared to our normal control group (p=0.0001). Macrosomic fetuses of nondiabetic (p=0.017) and diabetic women (p=0.0009) demonstrated larger CCD's than the control group. The CCD/BPD ratios of macrosomic fetuses were also different from the control group with higher significance in the diabetics (p<0.00001) than the nondiabetics (p=0.05). Subsequent to this study, diabetic mellitus was suspected in the third trimester in three women with no prior prenatal care, solely on the basis of high CCD and CCD/BPD ratios and later confirmed by chemical testing.
CONCLUSION:The CCD is an effective measurement in the evaluation of subcutaneous tissues and reflects nutritional status in fetuses with normal and abnormal growth.

613 **LONGITUDINALLY ESTABLISHED FETAL GROWTH CURVES BASED ON 6,048 ULTRASOUND EXAMS.** W. Cusick[X], A. Vintzileos, D. McLean, D.Nardi, Univ. of CT Health Center, Farmington, CT

Existing fetal growth curves, including common biometric parameters and weight estimation, have traditionally been constructed using cross-sectional data obtained from a limited number of patients. This retrospective study sought to develop longitudinal growth curves throughout gestation in a large number of patients followed by serial ultrasound evaluations at the University of Connecticut Health Center. Patients were referred for a wide range of indications. Singleton, well dated pregnancies without anomalies detected by ultrasound, were followed with serial ultrasounds throughout gestation. A total of 6,048 ultrasound evaluations were performed on 2,419 patients (mean exams/patient: 2.5, range 2-7). The following curves of commonly used ultrasound parameters were generated using computer assisted regression analysis including 5th, 50th and 95th percentiles.



The established normograms will assist the clinician in assessing fetal growth throughout gestation in a variety of high risk situations.

616 HUMERUS LENGTH MEASUREMENT AS A SCREENING METHOD FOR DOWN SYNDROME. D. Oberkrom[X], A. Fleming, D. Bonds[X], Dept. Ob/Gyn, Creighton Univ., Omaha, NE

In an attempt to diagnose Down syndrome (DS) by non-invasive means, we undertook the present study. FitzSimmons reported humerus length shortening in autopsy studies done on fetuses affected with DS. This was confirmed by antenatal sonographic studies performed by Benacerraf and Fleming. The purpose of this study was to see if sonographically determined humerus length could be used to identify fetuses with DS in patients at increased risk. Humerus length was measured in 60 patients with known risk factors for DS, including those with low maternal serum AFP, advanced maternal age, and a prior fetus affected with trisomy 21. Two humerus length (HL) measurements greater than 2 standard deviations below the mean established by Romero and Jeanty were observed. Of these, one had DS and the other, although genetically normal, had multiple congenital anomalies. Normal genetic outcome was noted in the 58 normal scans. These results support our previous findings and suggest that a HL measurement should be utilized as a screening method for DS.

618 **MATHEMATICAL MODELING OF FETAL WEIGHT PREDICTION**

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Previous studies suggested that birth weight (BW) prediction was enhanced by using formulas specifically derived from different gestational periods.In 440 pregnant patients between 24-40 weeks of gestation an ultrasound examination was performed within 72 hours of delivery. The following ultrasound parameters were considered: biparietal diameter(BPD), head circumference (HC),mean abdominal diameter (AD), abdominal circumference(AC), femur(FL) and humerus length(HL). Ultrasound measurements were correlated to fetal weight with multiple stepwise regression analysis in three different gestational periods: before 32 wks, 33-37 wks and 38-40 wks.The best mathematical models in predicting fetal weight in the different gestational periods were selected on the basis of the largest value of R^2 and the lowest mean unsigned and signed percent error : BW= 705.05-11.428 AC + 0.083 BPD^2 + 0.036 AC^2 + 0.244 FL^2 before 32 weeks; BW= -1374.397 + 0.128 BPD^2 + 0.198 AD^2 + 0.298 HL^2 between 33-37 weeks; BW= -5431.362 +19.17 BPD + 43.745 AD + 30.838 FL between 38-40 weeks. The accuracy observed during model development was confirmed during testing upon 809 non-model cases for the different gestational periods with an ultrasound examination obtained between 7 days of delivery(R^2=0.95; 0.90; 0.85 respectively). Comparison with other weight estimation procedures showed that our models gives weight estimates that are more accurate as those obtained with other methods proposed in the literature for different gestational ages.

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620 **Intrauterine Growth Retardation Overestimation by Ultrasound Predicted Estimated Fetal Weight.**

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Intrauterine growth retardation (IUGR) is a significant antepartum diagnosis that is associated with a marked increase in fetal and neonatal morbidity and mortality rates. Assuming an ultrasound estimated fetal weight less than the 10th percentile per gestational age, a diagnosis of IUGR was made. Clinical observation at delivery hinted towards an overestimation of the diagnosis in our population, based on birth weight criteria only. Between September 1987 and May 1991, 102 singleton pregnancies were identified as less than the 10th percentile estimated fetal weight (Acuson 128, OB calculation package). A retrospective review of maternal and neonatal records was undertaken. Parameters analyzed included maternal body mass index, amniotic fluid volume, fetal position, the number of ultrasound measurements obtained, placental grade, placental position, gestational age at the time of measurement and the interval between ultrasound measurements and delivery. A miscalculation of ultrasound predicted IUGR was identified as a birth weight greater than the tenth percentile. 28/102 (27.5%) were miscalculated (expected 12-20%). None of the individual factors analyzed were statistically significant in predicting a miscalculation. We conclude from these data that there are other factors influencing the ultrasound overestimation of IUGR in our population. Institutional derived birth weight curves correlated with the OB calculation package may allow for a more accurate prediction in our population.

621 **THE EFFECT OF GESTATIONAL AGE AND AMNIOTIC FLUID INDEX ON THE ABILITY TO VISUALIZE THE FETAL ABDOMINAL WALL CORD INSERTION BY ULTRASOUND**

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Fetal abdominal wall defects, especially omphaloceles, can be difficult to detect by ultrasound because of fetal trunk flexion, and/or small part crowding. This study was designed to investigate whether successful imaging of the umbilical cord at the fetal abdominal wall was related to gestational age and/or amniotic fluid index. Fourteen normal women were scanned every 2 wks from 20 wks to delivery. The amniotic fluid index was obtained and if the cord insertion into the abdomen could be visualized, the diameter was obtained. There were no maternal disorders, amniotic fluid, or fetal abnormalities. The mean birth was weight 3569 ± 169 grams.

WEEKS	CORD DIAMETER (cm)	# (%) VISUALIZED	AFI
20	9.2 ± 1.2	13/14 (92.9)	129.3 ± 30.7
22	10.1 ± 1.1	12/14 (85.7)	135.8 ± 33.7
24	11.2 ± 1.0	12/14 (85.7)	139.6 ± 24.5
26	12.3 ± 1.3	10/14 (71.4)	153.3 ± 26.7
28	14.0 ± 2.1	7/14 (50)	154.5 ± 34.6
30	14.3 ± 2.5	7/14 (50)	147.1 ± 30.9
32	15.3 ± 2.3	4/14 (28.5)	147.1 ± 24.8
34	14.0	1/14 (7.1)	156.0 ± 36.3
36	19.0	1/14 (7.1)	166.4 ± 35.6
38	18.0	1/14 (7.1)	156.7 ± 45.5
40		0/12	165.0 ± 36.2

We conclude that in spite of a trend toward a progressively increasing amniotic fluid index, the umbilical cord at the abdominal wall insertion becomes progressively more difficult to image. In fact, from 28 wks until term, the incidence of visualization well enough to obtain a measurement was less than or equal to 50%.

622 **SINGLE UMBILICAL ARTERY: IMPLICATIONS OF SONOGRAPHIC DIAGNOSIS**

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Eighteen cases of single umbilical artery (SUA) were diagnosed in utero over a 3 year period. There was one false positive diagnosis (at 18 weeks) and one patient is yet undelivered. Of the remaining 16 cases, 11 were referred for various obstetric indications, one for 2V cord, and 4 for fetal anomalies.

Eight patients had isolated 2V cord, including one with unexplained polyhydramnios. Each was correctly identified by sonography. Eight patients had identified associated anomalies; including one case each of Trisomy 13, Trisomy 18, Trisomy 21 and Turner Syndrome. The remaining cases were: VACTERL (2), holoprosencephaly (1), and omphalocele with bladder extrophy (1). In each case, sonography differentiated normal from abnormal babies, but in two of the anomalous infants, one or more major anomalies were missed by sonography. Based upon this experience, our current approach to the patient with SUA detected in utero is as follows: If careful sonographic evaluation shows no associated defects, we offer but do not recommend chromosomal studies. If other defects are seen, patients are counselled accordingly and amniocentesis is advised.

623 **GRAY SCALE AND COLOR DOPPLER ULTRASONOGRAPHY IN THE DETERMINATION OF AMNIOTIC FLUID INDEX**
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Concern that space occupied by the umbilical cord could be included inappropriately in measurements for amniotic fluid index (AFI) in pregnancies complicated by oligohydramnios led to an investigation comparing measurement of AFI by gray scale as opposed to color Doppler. Two hundred thirty-one patients (of whom 24 had oligohydramnios) were entered into the study. The general group of patients, as well as the sub-group with oligohydramnios, was found to have statistically lower AFI when measured by color Doppler than with gray scale alone ($P<0.05$). This finding was most important for the evaluation of patients with oligohydramnios. Conclusion: Use of color Doppler supplementing gray scale ultrasonography to determine AFI is more sensitive than gray scale alone for the prediction of oligohydramnios.

624 PRENATAL ULTRASOUND FINDINGS IN ASPHYXIATING THORACIC DYSTROPHY (ATD). O. Khawli, R. Morcos, M. Makii[X] Dept. Ob/Gyn, St. Elizabeth Hospital Medical Center, Youngstown, OH

Two cases of ATD (Jeune Syndrome) were diagnosed prenatally. Case #1: 30 year old G2 P1 has a child with Jeune Syndrome, referred because of a short femur at 36 weeks. The femur & humerus were < 5th percentile for gestational age. The thorax was bell shaped & on real time exams two weeks apart the fetus was in the same position, flat on the back with both thighs & knees flexed. The diagnosis of Jeune Syndrome was confirmed at delivery. Case #2: 34 year old G1 with negative family history, referred at 23 weeks because of short femur by ultrasound. At 16 wks, femur was at the 90th percentile. At 23 wks, femur was < 5th percentile & thorax was at 97th percentile. At 27 & 34 weeks, the femur & humerus were < 5th percentile (Rhizomelic Dwarfism). The thoracic circumference, which was > 50th percentile at 27 wks, fell below 5th percentile at 34 weeks. Diagnosis of Jeune Syndrome was suspected & confirmed after birth.
CONCLUSION: Prenatal diagnosis of Jeune Syndrome can be made by ultrasound based on the short femur length & a small thorax.

625 **ANTENATAL SONOGRAPHIC DIAGNOSIS OF DANDY-WALKER MALFORMATION IN THE LATE FIRST TRIMESTER.**
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The Dandy-Walker Malformation (DWM), characterized by complete or partial absence of the cerebellar vermis, cystic dilatation of the fourth ventricle and frequently hydrocephalus has been detected in-utero, but often after fetal viability. DWM is thought to originate in the 6th or 7th week of embryonic development but most case reports to date describe antenatal diagnosis at gestations averaging about 24 wks. Early prenatal diagnosis allows more time for thorough fetal anatomic evaluation and chromosome studies, thus increasing management options. We recently detected a cystic dilatation in the posterior fossa of a fetus at 12.5 wks. gestation (by LMP and crown rump length) along with probable cerebellar maldevelopment, using transvaginal sonography (TVS). Repeat TVS at 13.5 wks revealed dilatation of the fourth ventricle which appeared to communicate through the absent vermis with a posterior fossa cyst. Transabdominal CVS revealed a normal karyotype and no other anomalies were noted. The patient elected termination of pregnancy at 18 wks. via prostaglandin induction of labor. Evaluation by a fetal pathologist confirmed the DWM with a cystically enlarged fourth ventricle, absent cerebellar vermis and elevation of the tentorium and torcula. A 4mm round midline occipital defect was also found along with mandibular hypoplasia, cleft palate and limb contractures. Thus it becomes apparent that TVS may allow the antenatal diagnosis of DWM as early as 13 wks though problems remain regarding the prediction of its natural history in-utero as well as prognostication for postnatal life.

627 EFFECT OF LOW DOSE ASPIRIN ON MATERNAL AND FETAL FLOW VELOCITY WAVEFORMS. Peter Mc Parland,[X] J.M. Pearce,[X] Fetal Welfare Laboratory, St. George's Hospital, London, U.K.

Preeclampsia is associated with reduced uteroplacental and umbilical blood flow. The increased platelet activity and reduced placental flow that occur in this disorder may be due to thromboxane A2. Low dose aspirin (LDA) has been shown to reduce the incidence and severity of preeclampsia by inhibiting platelet thromboxane (TxA2), a potent inhibitor of vascular smooth muscle contraction and platelet aggregation whilst relatively sparing prostacyclin production. Thus it may be expected that LDA would improve blood flow resistance indices from the uteroplacental and umbilical circulations. As part of a randomised placebo controlled study evaluating the role of LDA (75mgs/day) from 24 weeks in high risk patients Doppler studies were performed in patients at 24-25, 27-28, 32-34 and 36-37 weeks.

Results

	Uteroplacental (RI)		Umbilical (S/D)	
Weeks	Placebo (SD) (n=26)	Aspirin (SD) (n=26)	Placebo (n=26)	Aspirin (n=26)
24-25	0.68 (0.08)	0.67 (0.05)	4.08 (1.2)	4.09(0.9)
27-28	0.62 (0.09)	0.57 (0.09)	3.72 (1.5)	3.32(0.8)
32-34	0.61 (0.08)	0.55 (0.1)	3.07 (0.8)	3.06(0.8)
36-37	0.53 (0.07)	0.52 (0.1)	2.57 (0.4)	2.64(0.5)

Although clinical benefit was seen in the aspirin group, no differences in resistance indices were seen between the 2 groups suggesting no significant vasodilatory effect of low dose aspirin

628 ABDOMINAL AORTIC TIME TO PEAK: AN INDEX OF FETAL MYOCARDIAL CONTRACTILITY. IM Bernstein, MC Meyer, Dept of Ob/Gyn, Univ. Vermont, Burlington, VT

We examined the fetal abdominal aortic time interval between initiation of systole and peak velocity systolic flow (time to peak) to establish measurement reproducibility and the normal values across gestational age. Sixteen subjects with AGA newborns were studied. We obtained an average of 5.2 sets of observations per subject between 21 and 39 weeks gestation. An ADR Ultramark IV (ATL, Bothell, WA) pulsed doppler was used. The abdominal aorta was insonated between the diaphragm and the aortic bifurcation during fetal apnea. A minimum of two waveforms, obtained from distinct angles of sonoincidence, with normal S/D were measured per observation. Intraobserver coefficient of variation with two observations was 12.6%. Interobserver coefficient of variation was 15.5%. Mean abdominal aortic aortic time to peak values demonstrated a significant positive linear relationship with gestational age (range; 0.045 sec to 0.072 sec). We believe that the examination of fetal abdominal aortic time to peak will assist in evaluating the contribution of myocardial dysfunction to the generation of abnormal fetal arterial waveforms.

629 **REOCCURRENCE OF UMBILICAL END DIASTOLIC FLOW THROUGH MATERNAL VOLUME EXPANSION**

Veronique H.M. Karsdorp MD*, John M.G. van Vugt MD PhD*, Gustaaf A. Dekker MD PhD*, and Herman P. van Geijn MD PhD*, Dept. of Obstetrics, Free University Hospital Amsterdam, The Netherlands.

Absent or reversed end diastolic (ARED) flow velocity waveforms in the umbilical artery are associated with poor fetal outcome. In the current study the effect of volume expansion on placental blood flow and neonatal outcome was investigated in 7 prenancies with ARED flow (group I). Seven pregnancies with ARED flow but conventional treatment served as controls (group II). In group I end diastolic flow completely reappeared temporarily after volume expansion, in the control group all cases continued to demonstrate ARED flow. In group I the overall survival rate was significantly higher (71%) than in group II (14%). There was no significant difference between the two groups with regard to birth weight or mean gestational age at delivery. No complications were seen with volume expansion. These preliminary data suggest that it is possible to temporarily improve uteroplacental circulation with volume expansion, resulting in a better neonatal outcome. It is suggested that this improvement may be based on an elevation of maternal-fetal oxygen and fluid exchange which may lead to a decrease of the oxygen free radicals mediated vasoconstrictive effect and/or improves local haemorrheologic conditions and/or a decrease in local uteroplacental angiotensin II production.

630 AGE-STANDARDIZED DOPPLER SD VALUES IN HIGH-RISK PREGNANCIES. T.Kaneoka, Y.Makino*, H.Izumi*, K.Shirakawa*, Fukuoka Univ. Sch. Med., Japan

All perinatal parameters depend on both gestational age and methods. In order to assess the clinical value of umbilical and uterine arterial Doppler PI values and other perinatal parameters to predict fetal outcome, all perinatal values obtained in 207 high-risk pregnancies were converted to age-standardized SD values based on our own standard values. As a result, it was found that the correlation coefficients of the umbilical PI values were 0.71 to NST, 0.64 to CST, 0.61 to fetal distress, -0.43 to plasma hPL, -0.39 to AC, and -0.37 to fetal body weight. The negative predictive values of the umbilical PI value (cut off value: 2SD) were 95% in NST, 89% in CST, 87% in fetal distress, 96% in low Apgar score and 85% in IUGR. The positive predictive values for fetal distress were 100% in intrapartum CTG, 45% in NST, 65% in umbilical PI and 52% in uterine PI, and those for IUGR were 96% in estimated fetal body weight, 57% in umbilical PI and 44% in uterine PI. However, the positive and negative predictive values of umbilical AEDV were 93% and 91%, respectively. It was concluded that age-standardized Doppler values were useful in the antenatal screening.

633 EXPECTANT MANAGEMENT OF HYPERTENSION IN PREGNANCY
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Aim: To assess the value of expectant management through the analysis of perinatal results. Method: Home bedrest if diastolic blood pressure (dBP) ≤ 99mmHg; hospitalization if dBP ≥ 100mmHg with single drug treatment if necessary. Interruption of pregnancy in cases of hypertensive emergencies and/or unresponsive eclampsia, severe intrauterine growth retardation and/or fetal distress. Material: 230 hypertensive pregnant women were studied: 126 with essential hypertension (EH) (G.1), and 104 with pregnancy-induced hypertension (PIH). Of these 104 PIH women, 34 only had hypertension (G.2), and 70 had preeclampsia (PE) (G.3). 115 normotensive pregnant women were used as controls. Results: Mean dBP in the 3rd trimester was 104.4 ±10; 98± 8 and 108±9mmHg in Groups 1, 2 and 3, resp. Mean gestational age (GA) at delivery was ≥ 38 wks in the 3 hypertensive groups. The rate of spontaneous delivery was 79 % in controls, 66% in G.1 and 56% in Gs. 2 and 3. Group 3 had the lowest mean birthweight (BW) as compared with non proteinuric PIH (G.2) (2691±749g vs 3119±718g). Low BW for GA was 50% and 29% for G.3 and G.2, resp. In EH, mean BW (3382±637g) was similar to that in normotensives (3344± 498); however, there was a lower rate of low BW for GA babies than in controls (9.5% vs 12.2%,resp.). Conclusion: Expectant management allowed for a decrease in preterm deliveries and an increase in spontaneous labor. EH per se was not related to poor perinatal prognosis.

634 **NIFEDIPINE IN THE SECOND-LINE TREATMENT OF SEVERE PRE-ECLAMPSIA REDUCES THE AMOUNT OF PROTEINURIA** Romanini C.[Y], Tranquilli AL.[X], Valensise H.[Y], Garzetti GG.[Y] Institute of Obstetrics & Gynecology, University of Ancona, Ancona, Italy

Nifedipine was assessed as a second-line treatment in 59 severe preeclamptic patients resistant to previous labetalol or methyl-dopa treatment. Nifedipine was added at a dose of 40-60 mg/day. Blood pressure was controlled and became stable at ≤ 90 mmHg diastolic within 48 hours, in 55 patients, allowing to prolong pregnancy (mean 12 days, range 3-45). The combination of nifedipine and labetalol reduced the amount of proteinuria by ≥ 25% in 15 patients. Nifedipine should be included in the treatment of severe pre-eclampsia and seems to protect against the evolution of proteinuria.

636 NISOLDIPINE : PRELIMINARY RESULTS USING A NEW ORALLY ADMINISTERED CALCIUM ANTAGONIST IN THE TREATMENT OF SEVERE POSTPARTUM PREGNANCY INDUCED HYPERTENSION (PIH). Michael Belfort.[X] Dept. OB/GYN, Groote Schuur Hospital, University of Cape Town, South Africa.

Calcium antagonists are frequently used to manage severe PIH. This study was designed to assess the clinical use of nisoldipine, a new, oral, long acting dihydropyridine, in severe postpartum PIH. MATERIALS AND METHODS: Nisoldipine (Bayer) was given orally (20mg, eight hourly) to 12 patients with severe postpartum PIH (MAP>126 mmHg: >3+ proteinuria). Blood pressure was continuously monitored using an arterial line. Continuous and intermittent 12 lead ECG monitoring was undertaken. Data analysis was with 1-way ANOVA($p<0.05$ was significant) RESULTS: Systolic ($p<0.01$) and diastolic ($p<0.01$) blood pressure fell within 30 minutes of initiation of therapy. Significantly reduced blood pressure was maintained with successive doses (q 8 hrs) for the 24 hr study period. There were no significant changes in the heart rate or ECG. There were no adverse reactions despite the high dosage. CONCLUSIONS: Nisoldipine rapidly, effectively and safely reduces blood pressure in severe postpartum PIH. The potential advantages of this oral calcium antagonist should stimulate further controlled investigation.

637 **MANAGEMENT AND OUTCOME OF SEVERE PRE-ECLAMPSIA IN 209 PRETERM PREGNANCIES.** John F. Rodis, Edward J. Wolf, Luanna Lattieri, Dennis Scribner[X], Winston A. Campbell, Anthony M. Vintzileos, University of Connecticut Health Center, Farmington, CT

In 1978, Zuspan stated "severe forms of preeclampsia are preventable and should never occur, but once present they should yield a zero maternal mortality rate and a fetal salvage of greater than 90 percent." We undertook this retrospective study of severe preterm preeclampsia over 10 years (1980 - 1989) to assess whether Zuspan's goals are achievable in a preterm population. All charts with a diagnosis of preeclampsia were reviewed. Severe preeclampsia was diagnosed in 209 preterm patients (21-36 weeks) based on the following criteria: BP≥160/110 (75%), cerebral symptoms (34%), epigastric pain (22%), severe proteinuria (63%), oliguria (1%), elevated liver enzymes (29%), elevated creatinine (28%), thrombocytopenia (29%), or intrauterine growth retardation (27%). Mean (±SD) maternal age 26.3 (±5.9)yrs; 71% were nulliparous, 81% were white , 88% had singleton gestations, mean gestational age was 31.9 wks(±3.0); and 89% were maternal transports. Management consisted of maternal stabilization and prompt delivery, either via oxytocin induction or cesarean section. IV $MgSO_4$ was used in 97% of cases. The cesarean section rate was 83%. The mean number of antepartum and postpartum days in the hospital were 1.9 and 5.7 respectively; 87% of patients were discharged ≤7 days of delivery. Only 5 patients (2.5%) were discharged with significant morbidity (1 cortical blindness, 2 Bell's palsies, 1 aphasia and 1 blurred vision); all resolved spontaneously. Of 238 infants, 23 % were SGA with mean birthweight of ± 1661 grams; and 93% were discharged alive. Our data suggest that with aggressive management (i.e. delivery), routine use of magnesium sulfate and the liberal use of cesarean section, Zuspan's goals of zero maternal mortality and greater than 90% fetal salvage rates are obtainable, even in preterm severe preeclamptics.

638 **ENDOTHELIN-1 SERUM LEVELS IN NORMAL AND PREECLAMPTIC PREGNANCIES.** C. Croom, MD[X], T. Nolan, MD[X], L.Devoe,MD, B.Lightfcot,MS[X], R.Caruana,MD.[X].Dept OBGYN and Nephrology, Med Coll Of Georgia,Augusta,Georgia

Endothelin-1(ET1) is a potent vasoconstrictor; its serum levels rise in response to endothelial cell damage. We studied 5 normal women before pregnancy and during all trimesters and labor to determine the course of ET1 levels. We studied 18 additional normal third trimester women before and during labor,8 term preeclamptic(PE) patients before and during labor,and 21 third trimester PE patients in active labor. Serum ET1 was determined by RIA. In normal pregnancy, mean ET1 levels rose significantly from 0.5 pg/ml(1st trimester) to 1.1 pg/ml and 1.7 pg/ml (third trimester and labor, respectively). Mean ET1 levels of PE patients were significantly higher than those of normal patients before labor (1.9 pg/ml vs. 1.2 pg/ml, p=.04) and during labor(3.5 pg/ml vs 1.6 pg/ml, p = .006).Mean ET1 levels in PE did not change significantly with labor(p=.07). The gradual increase in ET1 with gestational age and the differences in PE and normal patients are consistent with earlier reports. As the presence of labor and PE both lead to significant increases in ET1, the potential value of ET1 for discriminating PE from normal patients would be greatest before labor begins.

639 **PLACENTAL ABRUPTION AND RENAL DYSFUNCTION** SL Baker*, FL Gaudier, JC Hauth, SP Cliver. University of Alabama at Birmingham

Placental abruption severe enough to cause fetal death can result in acute renal failure or transient dysfunction. Over a seven year period (January 1983-June 1990), 37 patients with placental abruption and fetal demise were managed at our institution. Six of the women had renal dysfunction (one required dialysis on 7 occasions over 10 days), defined as a maximum serum creatinine ≥3.0 mg/dl. These women were compared to those whose creatinine remained <3.0 mg/dl (n=31). Both groups hac a similar maternal age and parity, gestational age, and birth weight. Their admission hematologic and coagulation studies and mean units of blood, other blood products, or crystalloid given in the first 6 or 12 hours were similar. The diagnosis-to-transfusion or delivery intervals were similar in each group. Serum creatinine and urine output (UOP) values were:

Serum Creatinines/Urine Output	Maximum Creatine <3.0 mg/dl	Maximum Creatine >3.0 mg/dl	P Value
Admission creatinine (mg/dl)	0.9	1.5	.06
Discharge creatinine (mg/dl)	0.9	2.2	.04
Maximum creatinine (mg/dl)	1.1	6.6	.03
Urine output-first 6 hrs (cc/hr)	53	21	<.01
Urine output-first 12 hrs (cc/hr)	65	33	.01

All six women with a maximum serum creatinine of ≥3 mg/dl had preeclampsia and thrombocytopenia (<100,000 platelets/mm3) versus 19 (60%) of those whose maximum creatinine remained <3.0. Hypofibrinogenemia (< 150 mg/dl) was similar in both groups. For both groups, the amount of crystalloid and blood infused was similar in the first 6 or 12 hours but the women with a subsequent creatinine of ≥3 had a significantly lower UOP during this interval, and eventually received more blood therapy. An analysis of the 37 patients based on a maximum creatinine of <1.4 or ≥1.4 mg/dl yielded similar results. We speculate that more prompt and adequate correction of intravascular volume may have ameliorated the renal dysfunction in these six women. However, we cannot be certain that the subsequent renal dysfunction had not been determined by events and timing that occurred prior to admission since these six women trended toward a higher creatinine level on admission.

640 **SINGLE DOSE ANTIBIOTIC THERAPY FOR CLINICAL CHORIOAMNIONITIS PRIOR TO VAGINAL DELIVERY.** C. Berry*, K.A. Hansen*, J.F. McCaul, Dept of Ob/Gyn, Naval Hospital, Portsmouth, Virginia.

Intrapartum antibiotics for clinical chorioamnionitis (CHOR) is well established treatment. Anecdotal and retrospective data suggest that vaginal delivery without postpartum antibiotics may be adequate therapy. We hypothesized that patients with CHOR who deliver vaginally do not benefit from postpartum antibiotics in the absence of persistent fever. 41 term laboring patients diagnosed with CHOR who subsequently delivered vaginally after a single dose of ampicillin and gentamicin were prospectively randomized. 21 received continuous antibiotic therapy and 19 were assigned a placebo in a double-blinded fashion. One subject in each arm had continued postpartum febrility ($p = 0.74$, by Fischer's exact test). We concluce that patients with CHOR who deliver vaginally can safely be observed for signs of persistent infection without continuing postpartum IV antibiotics.

641 **LEWIS ANTIGEN EXPRESSION IN WOMEN WITH PRETERM LABOR OR PRETERM PROM.** William F. O'Brien, German Leparc*, Jodi Holbrook*. Univ of South Florida, Tampa, FL

Lewis antigens are polysacharides produced by a number of cell types which are transported in the circulation by adsorption to red cell membranes. The inclusion of Lewis antigens into the cellular membrane interferes with the attachment of gram negative bacterial pili to the cell resulting in a natural defense against colonization in individuals who express the Lewis antigen. Expression of Lewis antigens has been shown to be an important risk factor in women with recurrent urinary tract infection with a higher frequency of non-expression (a-b-) in women with recurrent infection. In view of the importance of genital tract infection in preterm labor (PTL) preterm premature rupture of the membranes (PROM) we investigated the possible association of these complications with Lewis antigen phenotype as expressed on red blood cell membranes.

	White			Black		
LEWIS	PROM	PTL	Control	PROM	PTL	Control
a-b+	18 (60)	15 (75)	38 (58)	15 (42)	15 (56)	21 (38)
a+b-	7 (23)	3 (15)	16 (24)	6 (17)	3 (11)	9 (16)
a-b-	5 (17)	2 (10)	12 (18)	15 (42)	9 (33)	26 (46)

Although the incidence of women who failed to express Lewis antigens was significantly higher when compared to a non-pregnant population, when the results were adjusted for race there was no evidence of an excessive rate of a-b- women in the PROM or PTL groups. It appears that Lewis antigen expression and therefore bacterial attachment to vaginal epithelium is not an important component in the risk of PROM or PTL.

642 **PREVALENCE OF SEXUALLY TRANSMITTED DISEASE IN HIV SEROPOSITIVE PREGNANT WOMEN.** Sharon L. Patrick, M.D.* and Harold E. Fox, M.D., Department of Obstetrics and Gynecology, Sloane Hospital for Women, Columbia Presbyterian Medical Center, New York, NY

Pregnant women infected with the human immunodeficiency virus (HIV) are more likely to contract sexually transmitted diseases (STDs); the magnitude of this problem varies among populations. We compared the prevalence of STDs in pregnant HIV seropositive (HIV+) women with pregnant HIV seronegative (HIV-) and non-pregnant HIV+ individuals. In a retrospective case-controlled study, thirty-three pregnant women who were HIV+ underwent routine prenatal screening for syphilis, hepatitis B surface antigen (HBsAg) and PAP smear analysis. These women were matched for age and socioeconomic status with both pregnant HIV- women and non-pregnant HIV+ women. Results were analyzed using Chi square contingency table analysis. The HIV+ gravidas had a syphilis prevalence of 36% which was four times higher than the pregnant HIV- women ($p<0.05$). Pregnant HIV+ women had a higher prevalence of both HBsAg seropositivity (29% vs. 3%, $p<0.01$) and cervical intraepithelial neoplasia (CIN) on PAP smear (34% vs. 3%, $p<0.005$) when compared with pregnant HIV- counterparts. Similarly, HIV+ non-pregnant women had prevalence rates of syphilis (28%), HBsAg seropositivity (25%), and CIN (31%) which were significantly higher than their pregnant HIV- counterparts but not statistically different from the HIV+ pregnant cohort. HIV+ women, pregnant and non-pregnant, had significantly higher rates of STDs in our population. We conclude that factors other than pregnancy status contribute to the high prevalence of STDs among women infected with HIV. Thus, pregnant women with HIV infection require aggressive surveillance with regard to STDs.

644 UTILITY OF BLOOD CULTURES IN POSTPARTUM ENDOMYOMETRITIS. C. King,[x] P. Charache,[x] J. Repke, Depts. Gyn/Obs and Infect. Dis., The Johns Hopkins Univ. Sch. of Med., Balto., Md. 21205

This study was undertaken in an effort to evaluate the utility of blood culture information in the management of patients with postpartum endomyometritis. An 18 month period was evaluated during which positive blood culture results were reviewed. Variables included assessment of the appropriateness of antibiotic treatment before and after blood culture results were available, effect of blood culture results on antibiotic selection, and effect of blood culture results on duration of use of antibiotics. During this period of time, 16 confirmed positive blood cultures were reported, while an estimated total of 288 sets of blood cultures were obtained. Positive blood cultures, therefore, were present in 5.5% of patients with postpartum endomyometritis. Among these patients, there were no cases identified where additional antibiotics were necessary after blood culture identification and sensitivities were made available. There were eight cases where an antibiotic was judged to be superfluous based on blood culture results. In conclusion, given the high incidence of postpartum endomyometritis, the low incidence of blood culture positivity, and the broad spectrum antibiotics used in treatment, and given the cost of blood cultures ($28 per set), we recommend a reevaluation of the effectiveness of blood cultures, as currently utilized, in the management of postpartum endomyometritis in otherwise uncompromised healthy obstetric patients.

645 MULTICOMPARTMENT MOLECULAR IN UTERO EVALUATION FOR CONGENITAL HERPES SIMPLEX VIRUS (HSV) AND CYTOMEGALOVIRUS (CMV) INFECTIONS BY CHORIONIC VILLUS SAMPLING (CVS) AND POLYMERASE CHAIN REACTION (PCR). NB Isada, MP Johnson[x], SM Berry, R Whitley[x], W Britt[x], W Holzgreve, MI Evans. Ctr for Fetal Diagn & Ther, Hutzel Hosp, Wayne St U, Detroit, Mi., Institute für Humangenetik, Münster, FRG & Div Ped Infect Dis, Dept Peds, U Alabama, Birmingham.

Congenital infections are difficult to evaluate prenatally. CMV is associated with IUGR and CNS defects. Primary HSV may be teratogenic. PCR is a recently idescribed molecular genetic technique which amplifies minute amounts of genetic material. We used a multicompartment evaluation to assess all possible routes and sites of infection. Viral cultures, histology and viral-specific PCR were used to analyze the CVS sampla. Viral cultures and PCR were used to analyze the amniotic fluid (AF). Cordocentesis for CBC, immune globulins and liver enzymes were performed. We evaluated two gravidas for congenital infections. Patient 1 developed primary HSV with meningoencephalitis in thje first trimester. She had a negative evaluation that included CVS, amniocentesis and cordocentesis. Nested PCR primers encompassing HSV glycoprotein B region were used to analyze the CVS tissue. She delivered vaginally at term elsewhere and developed genital HSV 12 hours postpartum. The neonate developed HSV & required IV acyclovir. Concerns have been raised regarding time of acquisition of infection. Patient 2 was a health-care worker seropositive for CMV IgM. She had positive PCR in CVS and AF samples using primers from the conserved region of the gB gene. Shell vial cultures from CVS, amniocentesis and cordocentesis were neg. PCR on WBCs from cordocentesis was neg. She delivered at 37wks a 6 lb healthy male infant. We conclude that multi-compartment evaluation can be useful in excluding in utero infection and that the presence of viral genetic material does not necessarily indicate fetal morbidity.

647 GROUP B STREPTOCOCCUS DETECTION: COMPARISON OF RAPID IMMUNOASSAY AND CULTURE. Jeffrey S Greenspoon, M Morgan[x], Stuart G Smith[x], Regta L Greenspoon[x], Malcolm L Margolin[x] Depts. Ob-Gyn and Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, California

The reliability of an immunoassay, ICON Strep B Test[R] (Hybridtech, San Diego, CA), was assessed by comparison with the standard culture for Group B Streptococcus (GBS). A simple, rapid means to identify GBS colonized patients has been sought in order to permit selective and expeditious administration of chemoprophylaxis to the colonized patients and to avoid unnecessary treatment of those not colonized. At the time of evaluation for preterm or term labor, premature rupture of membranes, or antepartum surveillance, two vaginal swabs were simultaneously obtained from 174 patients. One swab was cultured using standard techniques for identification of GBS. Colonization was defined as light (1+ growth on culture plate), moderate (2+ or 3+), or heavy (4+). The second swab was used to perform the rapid test according to the manufacturer's recommendations. The prevalence of any GBS vaginal colonization was 10 of 174 (5.7%, 95% CI, 2.8% to 10.3%). Five of 174 (2.9%, 95% CI, 0.9% to 6.6%) had moderate or heavy colonization. Five of the 6 patients with false negative rapid tests had fewer than 20 colonies per plate.

	Culture			
Rapid test	Positive	Negative		
Positive	4	1	Sens. 40%	PPV 80%
Negative	6	163	Spec. 99.3%	NPV 96.4%

Previous reports whose study design distinguished parturients with light growth on culture from those with heavy growth noted that the infants of mothers with light colonization were less likely to develop EOGBS, although the risk was not zero. This test will be especially useful for identifying patients heavily colonized with GBS who are likely to benefit from timely chemoprophylaxis.

648 IMMATURE LECITHIN/SPHINGOMYELIN (L/S) RATIOS AND PERINATAL OUTCOME IN HIGH RISK PREGNANCIES. M.A. Harper, Dept. Ob/Gyn, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC

Nine hundred thirty-seven L/S ratios (single-dimension thin-layer chromatography) were reviewed from the three year period preceding the initiation of surfactant therapy in premature neonates in our institution. Six hundred eight were less than 2.5 with no phosphatidylglycerol. Of these, 104 singletons without congenital anomalies delivered within 72 hours from the collection of fluid [amniocentesis (n=42) or vaginal fluid (n=62)] for L/S determination and are the data set for this analysis. The values of the immature L/S ratios correlated significantly with length of hospital stay (r=-.41, p=.0001), total days on supplemental O_2 (r=-.25, p=.009), total days ventilated (r=-.29, p=.003). Seventy-one percent of babies with a L/S less than 1.0 required some respiratory support (O_2, CPAP, ventilator) compared to 28% of babies with a L/S between 2.0 and 2.5.

Conclusion: The absolute value of an immature L/S ratio can be helpful in predicting perinatal outcome in high risk pregnancies and therefore is of benefit in timing of delivery in these patients.

649 **IS TWIN PREGNANCY DESTINED TO BE A BIOLOGICAL DISADVANTAGE? A STUDY OF PERINATAL OUTCOME IN PRIVATE AND INDIGENT TWIN POPULATIONS.** J. Gandhi, W. Cohen, S. Yeh. Department of OB/GYN, Albert Einstein Medical Center/Temple University, Philadelphia and Albert Einstein College of Medicine, New York.

This report analyses perinatal outcome and contributing maternal factors in 132 indigent (A) and 199 middle class (B) women from the municipal and private services of the same institution. Mothers in group A were younger and of higher parity (age 25.4 vs 29.4, p<0.001, parity 2.4 vs 1.6, P<0.001). Hispanic and black women comprised 84% of group A and 52% of group B. 84% of the group A and 100% of the group B received prenatal care. First prenatal visit was later in A than B (24.7±7.4 vs 12±6 weeks, p<0.001). First prenatal visit was at ≤ 12 weeks in 5.3% of A and 61.6 % of B. 75% of twins in A and 90% in B were diagnosed prior to 28 weeks. Antenatal testing was performed in 61% of A and 67% of B. Lower mean birth weight in A (2167±823 gms vs 2449±710 gms, p<0.001) occurred as a result of more babies weighing <1500 gms (21% vs 9.4%). Mean gestational age at birth was lower in A (34.4±4.4 vs. 36.2±3.9 weeks, p<0.001) due to greater number of babies of <32 weeks (26.4% vs 7.1%). 40% of A and 20% of B babies required NICU care, with average NICU days being 9.3±20 in A and 2.3±9 in B. PNM was 53/1000 in A and 31/1000 in B, (p<0.001). This excess PNM and morbidity of twins in the municipal sector was accounted for by the excess number of preterm babies born prior to 32 weeks and weighing <1500 gms. This adverse perinatal outcome was not associated with increased maternal complications, IUGR or late gestational age at diagnosis. Early prenatal care rather than early diagnosis appeared to have an association with better outcome. **In conclusion,** these data suggest that preventing premature labor prior to 32 weeks in twin gestation in indigent patient may be important in reducing extreme preterm birth and associated perinatal mortality and morbidity.

650 **COCAINE USE IN PREGNANCY: THE EFFECTS OF RESIDENTIAL TREATMENT ON PERINATAL OUTCOME.** Isaac Delke, Drew Leavitt[x], Luis Sanchez-Ramos, Mark T Cullen. University of Florida, Jacksonville, FL.

The perinatal impact of residential treatment of cocaine-using gravida is little known. The purpose of this study is to evaluate the effects of a comprehensive residential treatment program on the perinatal outcome among cocaine-using pregnant women. During an 18-month period, 394 cocaine-exposed infants were reported to Florida Health and Rehabilitative Services from Duval County. Data were available on 320 cases: Group 1 (N=39, residential treatment + prenatal care), Group II (N=138, prenatal care only), and Group III (N=143, no care). Maternal, obstetrical and neonatal data were collected from medical and drug treatment records. The statistically significant differences between the groups included: STDs, gestational age at delivery, preterm birth, birth weight, low birth weight (<2500 grams), small for gestational age, microcephaly (HC<10%ile), Apgar score at one minute, NICU admission and positive urine toxicology at birth. The statistically significant differences between groups I and II were: Low birth weight (p<0.01), microcephaly (p<0.02) and positive urine toxicology at birth (p<0.0001). **Conclusion:** Residential treatment contributed to reduction in low birth weight and cocaine-exposed infants at birth.

651 MANAGEMENT OF PREMATURITY: THRESHOLD FOR OBSTETRICAL INTERVENTIONS BASED ON NEONATAL OUTCOMES. R.T. DePalma, M.D., K.J. Leveno, M.D., M.A. Kelly,[x] M.L. Sherman,[x] UT Southwestern Medical Center at Dallas

We sought to determine the preterm birthweight for which obstetrical interventions intended to delay delivery might potentially improve neonatal morbidity and mortality. 550 singleton and twin livebirths weighing 1000 to 2199 grams and delivered at Parkland Hospital from January 1990 through May 1991 were retrospectively analyzed. The only pregnancy complications included were spontaneous preterm labor and preterm rupture of the membranes. Neonatal mortality and several indices of morbidity are summarized below:

Birthweight (grams)

	1000-1299	1300-1599	1600-1899	1900-2199
No. of infants	58 (%)	87 (%)	142 (%)	263 (%)
Deaths	6 (10)	4 (5)	0 (-)	0 (-)
Resp. Distress Syndrome	23 (40)	23 (26)	8 (6)	4 (2)
Intraventricular Hemorrhage	7 (12)	3 (3)	0 (-)	0 (-)
Necrotizing Enterocolitis	4 (7)	7 (8)	5 (4)	4 (2)
ICN days Mean (SD)	18.1 (24.7)	5.8 (11.5)	1.6 (3.7)	0.4 (1.3)

The table includes only respiratory distress requiring mechanical ventilation and Grade 3 or 4 intraventricular hemorrhage. Conclusion: These results suggest that there are few significant potential benefits in attempting to delay delivery of fetuses weighing 1600 grams or more.

652 VAGINAL POOL PHOSPHOLIPIDS AND NEONATAL OUTCOME. JA Spinnato, CA Marcell,[x] KK Ralston,[x] ER Greenwell,[x] JA Spinnato, III,[x] OB/GYN Dept., Univ. of Louisville School of Medicine, KY

The Lecithin/Sphingomyelin (L/S) ratio and phosphatidylglycerol (PG) value (chloroform extracted-acetone precipitated thin layer chromatography) were assessed on vaginal pool (VP) amniotic fluid (AF) specimens (n=106) from patients with preterm (<37 weeks) rupture of membranes (ROM) and compared to AF values obtained transabdominally (TA) (N=153) from non-diabetic control patients with intact membranes. Neonatal outcomes were compared for patients delivering within one week of AF assessment. Delivery was promptly accomplished in the ROM group if the LS ratio was ≥2 (43/45 within one day) and glucocorticoids were not used in those cases. Between 28 and 32 weeks' gestation the L/S ratio (1.9 ± 0.8, N=56) and PG (13/55 = Present) in the VP group were more mature than in the TA group (L/S = 1.3 ± 1.1, N=41, p<0.01, PG=present, 1/31, p<0.05). After 32 weeks' these differences were not observed. Among patients with an L/S ratio ≥2.0, hyaline membrane disease HMD was noted in 2/45 patients in the VP group versus 0/53 patients in the TA group (P=NS). HMD was not observed in either group (VP=0/23, TA=0/27) when PG was present. Ventilatory support for non-HMD diagnoses was briefly and infrequently required in both groups and no deaths or serious complications occurred when PG was present or L/S ≥2. False negative LS and PG were similarly frequent in both TA and VP groups. The L/S ratio and PG from VP predict neonatal pulmonary performance as well as when obtained TA. The ease of specimen collection and importance to clinical management encourages their use. The neonatal outcomes in this study support prompt delivery when ROM and positive maturity tests coexist.

653 PRETERM RUPTURE OF MEMBRANES BEFORE 25 WEEKS: PERINATAL OUTCOME

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Expectant management of PROM before 25 weeks has recently been advocated. An 8 year review of records at our facility reveal 17 cases in 10,500 admissions for an incidence of 0.16%. Six patients elected termination of pregnancy. Of the remaining eleven patients who chose continuation of pregnancy, 4 perinatal deaths occurred for a perinatal mortality rate of 364/1000. Mothers of surviving infants were older (25.3+/-4.5 vs. 21.3+/-3.3 years p=.025) and had higher gravidity (2.43+/-0.79 vs 1.5+/-0.58 pregnancies (p=.025) than mothers of non-surviving infants. There was no difference between survivors and nonsurvivors in gestational age at PROM (20.4+/-4.03 vs. 19.5+/-3.51 weeks), interval from PROM to delivery (12.8+/-5.69 vs 8.75+/-6.38 wk), and delivery weight (1788+/-356 vs 1600+/-453 gm) (p=.10). Neonatal morbidity included pneumonia (4), RDS (5), pneumothorax (1), persistent fetal circulation (1), pulmonary hypertension (3), sepsis (2), IVH (1), limb contractures (3). Expectant management may be offered, if patients are cautioned that it is currently not possible to predict which infants will survive prolonged PROM, and that there may be significant perinatal morbidity.

654 ELEVATION OF TOTAL PLASMA CORTISOL WITH PROM

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The role of cortisol in the onset of term or preterm labor in humans is still unknown. Total plasma cortisol (C_T) was determined for patients with preterm premature rupture of the membranes (PPROM) without labor, relative to historical controls (normal patients not in labor, gestationally matched). The gestational dependence of C_T was studied at the time of PPROM, and longitudinally for each patient following that event. Venous C_T obtained at 8 A.M. was assayed by RIA. (10 patients with PPROM between 23-35 wks; 6 serial determinations). All pregnancies were singleton, not in preterm labor, had no evidence of infection, and none were given glucocorticoids to induce fetal lung maturity. A mean of 2.4 days transpired from the last C_T determination to the onset of labor. RESULTS & CONCLUSIONS: (1) C_T was elevated for all patients with PPROM (mean C_T=48.2± 16.2 µg/dl; control C_T=32.0± 3.3 µg/dl), which is significant ($p<.0005$). (2) C_T values appear to be independent of the gestation at which PROM occurs, and serial values show no tendency to increase as long as labor does not ensue. (3) The rise in C_T may reflect the process in parturition.

655 **DECREASING CESAREAN SECTION RATES IN VERY LOW-BIRTH WEIGHT INFANTS: EFFECT ON PERINATAL OUTCOME**

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There is little evidence that the use of cesarean section for the delivery of very low-birth weight infants improves overall survival. Our specific objective in this study was to determine within the very low-birth weight category (500-1499 gms) whether a reduction in cesarean deliveries was associated with an increased risk of adverse perinatal outcome. The data used in this analysis were obtained from a review of 23,529 livebirths delivered at our institution from 1986 to 1990. Examination of these data revealed a significant decrease in the use of cesarean section for very low-birth weight infants from 55% to 40% ($p<0.05$) during the five year period. Concomitantly, the cesarean section rate for all birth weight categories decreased from 27% to 8% ($p<0.0001$). The neonatal death rate in the very low-birth weight category decreased from 33/1000 to 18/1000 ($p<0.01$). There was no significant change in the incidence of low Apgar scores, cord blood gas values, intraventricular hemorrhage, and median length of stay in NICU. Our results suggest that the cesarean delivery rate can be significantly reduced in very low-birth weight infants without adversely affecting perinatal outcome.

656 LAMELLAR-BODY NUMBER DENSITY PREDICTION OF FETAL LUNG MATURITY.

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The reliability of amniotic fluid lamellar-body number density (LBND) for the identification of the immature fetus was determined. The outcome predicted by LBND was compared to the clinical outcome of 15 infants delivered within 72 hours of amniocentesis. LBND was compared to other fetal lung maturity (FLM) tests performed on 65 samples. Lecithin-sphingomyelin (L/S), phosphatidylglycerol (PG), absorbance at 650 nm (A_{650}), and the foam stability index (FSI) were determined by standard methods. LBND was measured by resistive-pulse counting of uncentrifuged amniotic fluid (Dubin SB *Clin Chem* 1989;35:612). A "positive" FLM test predicts the development of hyaline membrane disease (HMD). A "negative" test predicts the absence of HMD. The sensitivity (Sens), specificity (Spec), positive predictive value (PPV), and negative predictive value (NPV) of LBND are shown in the table. The relation of LBND <40,000/microL to "positive" (immature) FLM test results is also shown.

LBND <40,000/microL	Clinical HMD	L/S <2	PG <3%	A_{650} <0.15	FSI <47
Sens n(%)	2/3 (67)	12/27 (44)	17/33 (52)	6/9 (67)	7/16 (44)
Spec n(%)	11/12 (92)	33/38 (87)	32/32 (100)	44/53 (83)	20/21 (95)
PPV n(%)	2/3 (67)	12/17 (71)	17/17 (100)	6/15 (40)	7/8 (88)
NPV n(%)	11/12 (92)	33/48 (69)	32/48 (67)	44/47 (94)	20/29 (70)
Prevalence of immature result n(%)	3/15 (20)	27/65 (42)	33/65 (51)	9/62 (15)	16/37 (43)

Every FLM had one clinical HMD false negative (FN) result; A_{650} had 2 FN results. LBND is similar to other FLM tests, but can be measured immediately on uncentrifuged amniotic fluid without interference from blood or pigments.

657 CLINICAL CHARACTERISTICS AND OUTCOME OF PATIENTS WITH RESEALED, PRETERM, PREMATURE RUPTURE OF MEMBRANES. S.J. Carlan, W.F. O'Brien, J.L. Glock[x], U of S Fl, Dept. OB/GYN, Tampa, FL.

From March 1, 1989 to March 1, 1991, a total of 386 women (2.5% of total number of deliveries) with preterm, premature rupture of membranes (PPROM-rupture of membranes prior to labor before 37 weeks gestation) were admitted to Tampa General Hospital. Nineteen women electively terminated the pregnancy, and, of the remaining 349 patients, 14 (4%) resealed. All patients with rupture were documented with a positive history of a gush of fluid from the vagina, and either fern, nitrazine, pooling or a combination. All patients classified as "resealed" reported that the leaking stopped and then were confirmed with amniocentesis and injection of indigo carmine. If no seepage of blue dye was noted from the vagina, they were classified as "resealed". There was no significant difference between the groups in racial makeup, GTPAL, or positive cervical cultures for Gc, Chlamydia, or GBBS. There were no multiple gestations in the resealed group and nine in the group that did not seal.

CLINICAL CHARACTERISTICS OF RESEALED VS SEALED (±1 SD)

	ReSealed (N=14)	Not Sealed (N=335)	P
Age (mean years)	20.0 ± 3.6	23.9 ± 6.2	<.05
EGA at ROM (mean weeks)	29.6 ± 4.6	31.7 ± 3.8	<.05
Hx previous PPROM (%)	14.3	10.1	NS
Initial US deepest pocket (mean cm)	4.6 ± 4.6	2.9 ± 1.6	<.05
ROM to delivery (mean d)	67.1 ± 37.6	5.3 ± 10.3	<.05
EGA at delivery (mean weeks)	38.6 ± 1.3	32.8 ± 3.7	<.05
C-Section (%)	14.3	16	NS
Neonatal wt (mean grams)	3280 ± 454	1952 ± 683	<.05
Cord pH (mean)	7.28 ± .08 (N=12)	7.3 ± .08 (N=280)	NS
NICU admissions (%)	0	52.8	<.05
Total neonatal days in hospital (mean)	2.4 ± 0.8	17.6 ± 23.1	<.05

We conclude that the patients that are more likely to reseal are younger and at an earlier gestational age and have larger pockets of amniotic fluid on initial ultrasound. After resealing the pregnanies apparently procede normally with no higher incidence of re-rupture.

658 **PREVIOUS SPONTANEOUS AND INDICATED PRETERM BIRTH AS RISK FACTORS FOR PREMATURITY**

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We reviewed the obstetric history of 33,430 women who were evaluated at the time of the March of Dimes Preterm Birth Prevention Project. As the number of prior PTDs (due to any etiology) rose from 0 to 1 to ≥2, the rate of PTD ≤37 weeks rose from 10 to 21 to 31% (p≤.0001). Similarly, the rate of PTD ≤34 weeks rose from 5 to 13 to 20% (p≤.0001). We then evaluated the risk of PTD as related to the etiology of the prior PTD. As prior spontaneous preterm delivery (SPTD) (following labor or PROM) rose from 0 to 1 to ≥2, the rate of SPTD ≤37 weeks in the current pregnancy rose from 7 to 17 to 26% (p≤.0001) and the rate of SPTD ≤34 weeks rose from 4 to 10 to 17% (p≤.0001). As the number of prior indicated PTD (IPTD) increased from 0 to 1 to ≥2, the rate of IPTD in the current pregnancy ≤37 weeks rose from 2 to 11 to 28% (p≤.0001) and the rate of IPTD ≤34 weeks rose from 2 to 7 to 24% (p≤.0001). One or more prior IPTD did not predict SPTD. Of 445 women with twin gestations, 53% delivered ≤37 weeks and 35% delivered ≤34 weeks. However of women with twins and 1 or more prior SPTD, the rate of SPTD ≤37 weeks was 53% (NS) and ≤34 weeks was 44% (p≤.03). This data will enable those planning studies of PTD intervention strategies to choose groups at highest risk for PTD and allow more precise power calculations.

659 **ESTIMATION OF FETAL WEIGHT BY ULTRASOUND IN PRETERM PREGNANCIES**

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Estimation of fetal weight (EFW) or gestational age in the preterm pregnancy often directs management of high risk patients. Mode of delivery of preterm breech presentation is currently based on EFW; aggressive tocolysis may be limited by EFW and even, consideration of operative intervention requires an assessment of age or EFW. These difficult decisions are most often made in a Labor and Delivery setting by physicians of varying expertise with ultrasound on patients with minimal historical information to allow gestational dating. This study determined the accuracy of fetal weights calculated from ultrasound measurements using the Shepard and 13 other formulas. 172 neonates whose birth weights were < 2000 gm were prospectively evaluated prior to delivery; all had ultrasound measurements (BPD, HC, AC, FL) within 7 days of delivery. The accuracy of each formula was assessed by a ratio of Estimated Fetal Weight(EFW) to Actual Birth Weight (ABW) and 95% Confidence Intervals. Only 3 formulas had 95% CIs that included 1.00, i.e. EFW not significantly different from ABWs: Hadlock #3, 1.004 ± 0.019; Hadlock #4, 1.010 ± 0.099; Woo #2, 0.992 ± 0.019. Errors tended to be exaggerated at ABWs < 500 gm. EFW significantly altered clinical management in a significant number of cases.

660 LONG-TERM INTRAVENOUS TOCOLYTIC THERAPY. J. Bruner, A. Bruner,* A. Sarno, Dept. of OB/GYN, Vanderbilt Univ. Medical Center, Nashville, Tennessee.

Eighteen women in preterm labor who received continuous intravenous tocolytic therapy for greater than 48 hours (108.6 ± SD 156.9 hrs; range 48.5-729 hrs) were compared to a similar group of women treated for less than 48 hours (12.9 ± SD 6.7 hrs; range 4-38.5 hrs) in this retrospective case-control study. The groups were well-matched in regard to age, race, weight, gravidity, parity, marital status, social status, level of prenatal care, and prior medical and surgical diagnoses. The only identifiable risk factors for preterm labor significantly associated with an increased need for long-term intravenous tocolytic therapy were uterine and cervical anatomic defects (fibroids, prior conization, incompetent cervix) and multiple gestation. The mean gestational age at the time of diagnosis was 30 weeks for both groups, and there were no significant differences in mean cervical dilatation or effacement on initiation of therapy. Tocolytic selection was similar in both groups, although the dosage/hr was significantly greater with long-term therapy. The mean interval from initiation of therapy until delivery was 41 days in the study group, compared to 39 days among controls (NS). The mean gestational age at delivery was 37 weeks in both groups. There was no significant difference in the incidence of fetal distress, mode of delivery, or neonatal Apgar scores. No statistically significant maternal or neonatal complications were noted in either group. These data demonstrate that long-term tocolytic therapy is a safe and efficacious means of prolonging gestation in those women who fail to respond to conventional treatment.

662 COMPARISON OF MAGNESIUM AND NIFEDIPINE FOR PRIMARY TOCOLYSIS AND IDIOPATHIC PRETERM LABOR. R.C. Floyd,[X] B.N. McLaughlin,[X] R.W. Martin, W.E. Roberts, W.L. Wiser,[X] J.C. Morrison, Dept. Ob/Gyn, Univ. Mississippi Med. Ctr., Jackson, MS

Objective: To compare nifedipine (N) with magnesium (M) as primary tocolytic agents for idiopathic preterm labor (PTL). Patient Population: In this prospective study, 67 patients between 20 and 34 weeks' gestation with documented PTL over a 12-month period were randomized to receive 2 mg of oral N q 8 hours versus intravenous M (sulfate) followed by oral M (gluconate), 2 gm q 4 hours orally. The diagnosis of PTL was established by repetitive contractions usually q 5 minutes with documented cervical change from a previous exam or cervix > 2 cm/> 50% effaced. Multiple gestation, chorioamnionitis, rupture of the membranes, fetal distress, growth retardation, or allergy to M or N were exclusion factors. Main Outcome Measured: Pregnancy prolongation index (days prolongation from diagnosis of PTL/ideal prolongation [to 37 weeks] from PTL), number delivering < 37 weeks, birth weight, maternal complications for tocolytics and duration to delivery after treatment. Results: There was no difference in the number of patients delivering < 37 weeks, the duration of treatment, interval to delivery, PPI, PTB, or BW.

Group	Number	Treatment Interval* (weeks)	PPI*	PTB*	BW* (gm)
M	29	7.0 ± 5.2	0.96 ± 0.55	11	2565 ± 763
N	39	5.7 ± 3.9	0.97 ± 0.59	13	2768 ± 662

*not significant

Conclusions: The results of this study show that N is a useful agent as a first-line tocolytic with an effectiveness comparable to that of M. No evidence of fetal or neonatal compromise was noted and there was no statistically significant difference between pregnancy prolongation or preterm births.

663 UMBILICAL BLOOD SAMPLING. IS IT IMPORTANT WHERE TO SAMPLE ? B. Peskin[X], N. Lazebnik, J. Blankstein[X]. Dept. Ob/Gyn, Case Western Reserve University, Mt. Sinai Med Ctr, Cleveland, OH.

Umbilical cord blood sampling at birth greatly enhances assessment of the newborn's respiratory status. This study was conducted in order to address two questions. 1) Does the cord blood gas result depend on the cord segment sampled ? 2) Does fetal distress play any roll in choosing the cord segment to be studied ? Three groups of patients were studied with 10 patients in each group. Group I Elective cesarean section with apgar score of > 9 at 1 and 5 minutes. Group II Normal spontaneous vaginal deliveries with no evidence of fetal stress as shown by fetal heart tracings and apgar score of > 7 at 1 and 5 minutes. Group III newborns delivered by forceps / vacuum extractor / cesarean section due to abnormal fetal heart tracings and scalp pH sample of < 7.20. For each group the cord was clamped immediately in 3 different sites. Near the placenta, mid portion and about 5 cm from the newborn. Blood gas samples within sites in the same newborn and between the 3 groups were compared by analysis of variance (ANOVA). The 3 groups were statistically different in cord artery pH results regardless of the site sampled (7.27+ 0.049 vs 7.26 + 0.033 vs 7.17 + 0.06 $p < 0.001$ for groups I, II , and III respectively). In groups I and II where no fetal distress was present no difference was found in cord artery pH between the different sampling sites. However in group III where biochemical evidence of fetal distress was present a significant difference was found in the arterial pH between the three sites sampled. In the site closer to the newborn the mean pH was 7.17 + 0.06, in the mid section of the cord the pH was 7.18 + 0.067 and in the site near the placenta the cord pH was 7.21+ 0.079. The same was found when the pCO2 was studied . Ana ysis of the the cord vein blood gases showed significant differences between groups but no difference within groups. In conclusion, the site sampled may play a roll in documenting the respiratory status if acidosis is present.

664 CLINICAL APPLICATION OF THE KLEIHAUER-BETKE TEST. A.R. Dupre,[X] J.N. Martin, Jr., R.C. Floyd,[X] P.G. Blake,[X] W.E. Roberts, J.C. Morrison, Dept. Ob/Gyn, Univ. Mississippi Med. Ctr., Jackson, MS

Objective: Determine the accuracy of quantitation of fetomaternal hemorrhage by Kleihauer-Betke (KB) testing to predict the maternal/neonatal outcome. Population: All women > 20 weeks' gestation at risk for fetomaternal hemorrhage (abdominal trauma, cocaine ingestion, placenta previa, and abruptio placenta) were consecutively evaluated in the labor and delivery suite. A case series study design was utilized. The analysis of maternal whole blood for the presence of fetal cells was accomplished by the KB procedure using a commercially available kit (Sure-Tech Diagnostics, Inc.). If positive, it was repeated at 6-hour intervals (if the patient remained undelivered) for 24 hours. Main Outcome Measured: The presence or absence of abruption at delivery, the estimated gestational age, as well as neonatal hematocrit, weight, pH, and Apgar score were assessed in women who had positive and negative KB tests. Results: Seventy-six patients were enrolled during the study period and a total of 109 KB tests were performed.

	Abdominal Trauma	Placenta Previa	Substance Abuse	Suspected Abruption
N	37	8	15	16
+ KB	4	2	5	7
+ Abruption	0	1	1	5
NN Hct < 45	0	0	0	1

The presence of a positive KB test was not of assistance in identifying significant fetomaternal hemorrhage. Neonatal (NN) outcome was good. There was no correlation between the KB tests either initially or on serial examination with Apgar scores or cord blood pH. Of the 18 patients with positive KB tests, only 1 infant had a hematocrit of < 45%. Even when positive, the amount of estimated fetomaternal bleeding by KB testing did not correlate with any outcome parameter. Conclusion: Significant fetomaternal bleeding or neonatal morbidity is not predicted by a positive KB test.

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Artist's enhancement of scanning electron microscopy: *C albicans* 24 hours posttreatment with butoconazole nitrate.*

When you diagnose vulvovaginal candidiasis (VVC)

FEMSTAT® Prefill (butoconazole nitrate) Vaginal Cream 2% is clinically effective against a broad spectrum of important pathogens, including *Candida albicans* and other *Candida* species, such as *C tropicalis.*



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1. Droegemueller W, Adamson DG, Brown D, et al. Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol.* 1984;64:530-534. *Micrographs on file, Syntex Laboratories, Inc. †In pregnant patients (second and third trimesters only), therapy should be extended to 6 days. Please see adjacent page for brief summary of prescribing information. © 1991 Syntex Laboratories, Inc. 813-12-196-91

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BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

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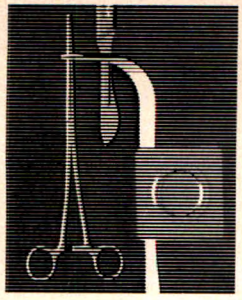
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
(FOR FULL PRESCRIBING INFORMATION, SEE PACKAGE INSERT.)

INDICATIONS AND USAGE
Treatment: CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary Tract Infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis,* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), *E coli, Proteus mirabilis,* and *Serratia marcescens.**
Skin and Skin Structure Infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *E coli, Klebsiella pneumoniae,* and *Peptococcus** and *Peptostreptococcus* sp.*
Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), group B streptococci, *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, and B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species).
Intra-abdominal Infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B distasonis, B ovatus,* and *B thetaiotaomicron*), and *Clostridium* species.*
Bone and Joint Infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.
Note: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
Prophylaxis: The preoperative administration of CEFOTAN may reduce the incidence of certain post-operative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS
General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.
Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.
Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
Drug/Laboratory Test Interactions: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.
Usage in Pregnancy: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
Usage in Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.
Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

During postmarketing experience with CEFOTAN, agranulocytosis, anaphylactic reactions, fever, hemolytic anemia, leukopenia, prolonged prothrombin time with or without bleeding, pseudomembranous colitis, and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION
Treatment: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

Prophylaxis: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.
Impaired Renal Function: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

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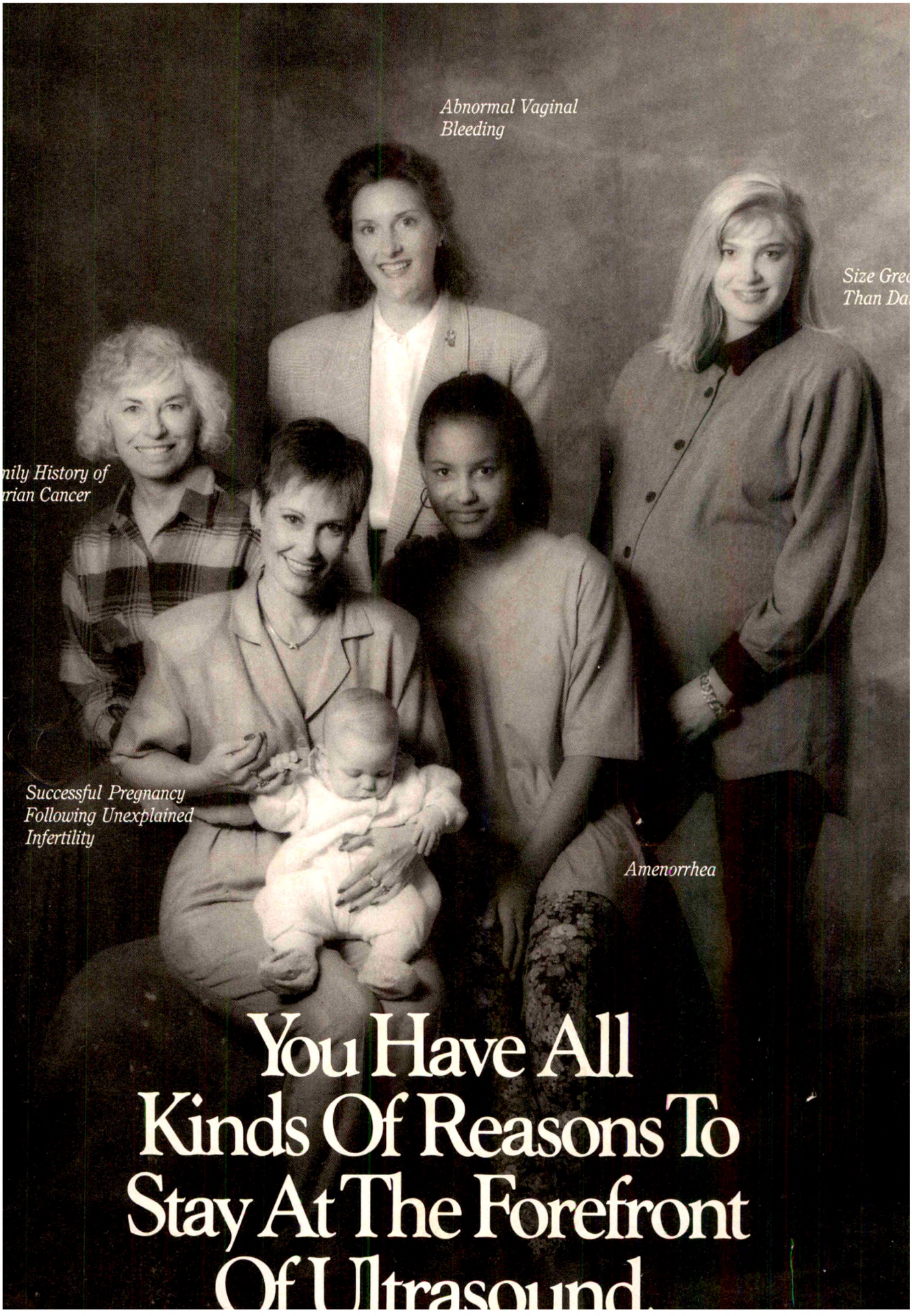
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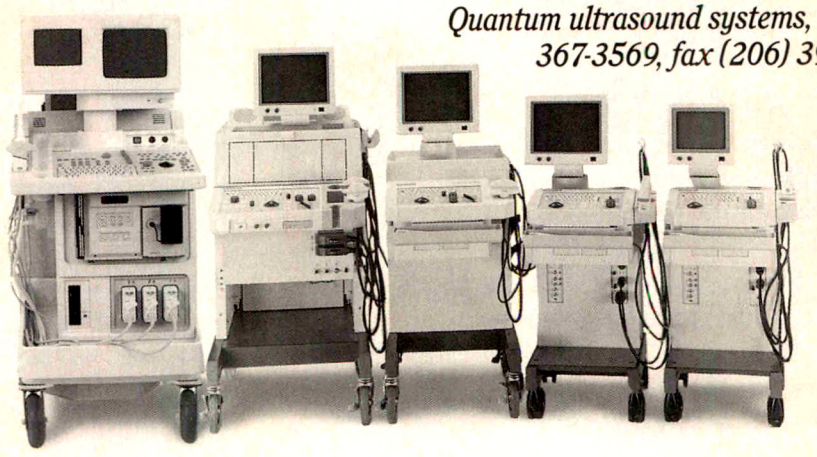
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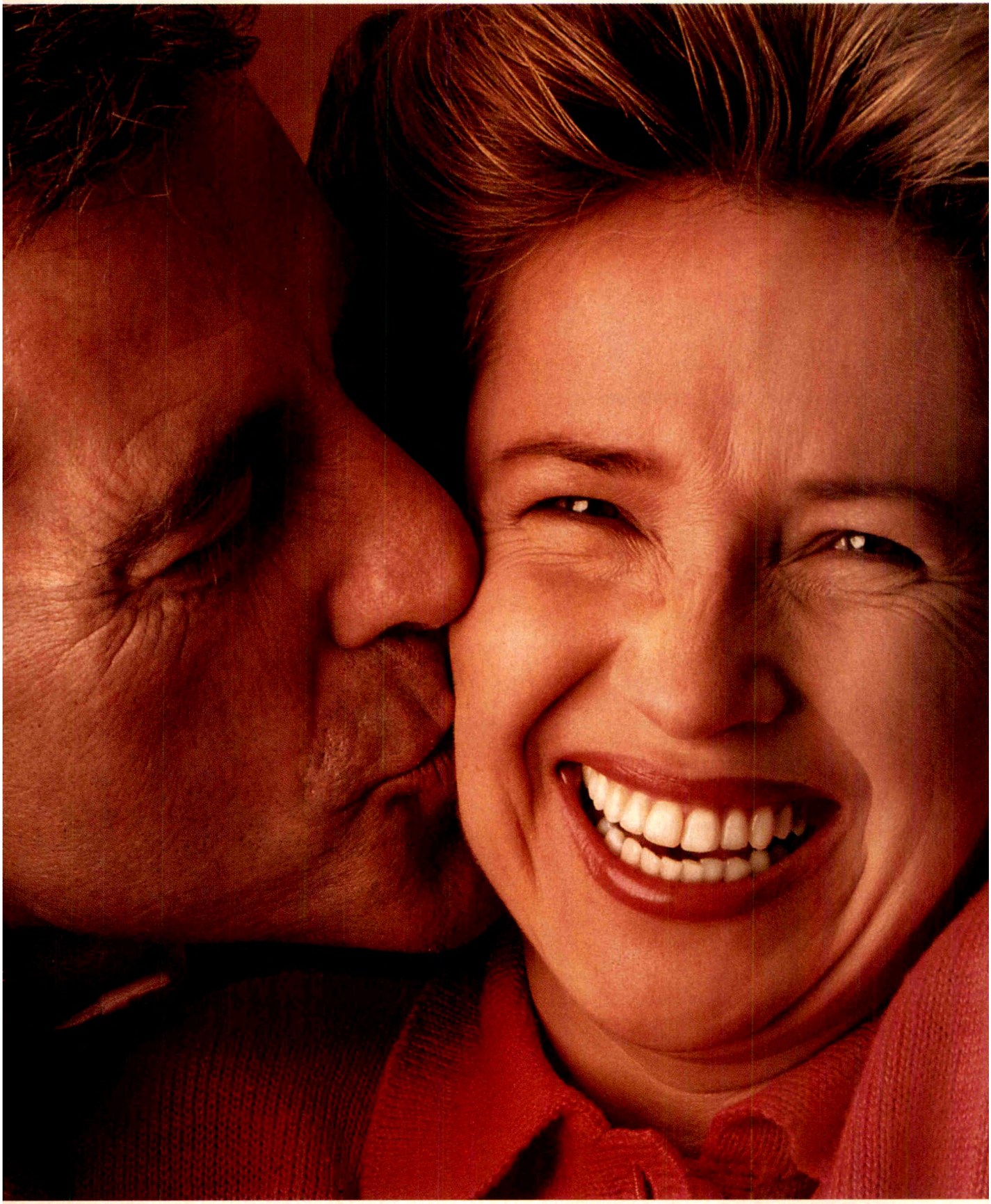
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Note: Estrogens have been reported to increase the risk of endometrial carcinoma in postmenopausal women.



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Please see brief summary of prescribing information on last page of this advertisement.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.
There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risks, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy: changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness; mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.
Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.
Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

Reference: 1. Weiss NS, Ure CL, Ballard JH, et al: Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. *N Engl J Med* 1980;303:1195-1198.

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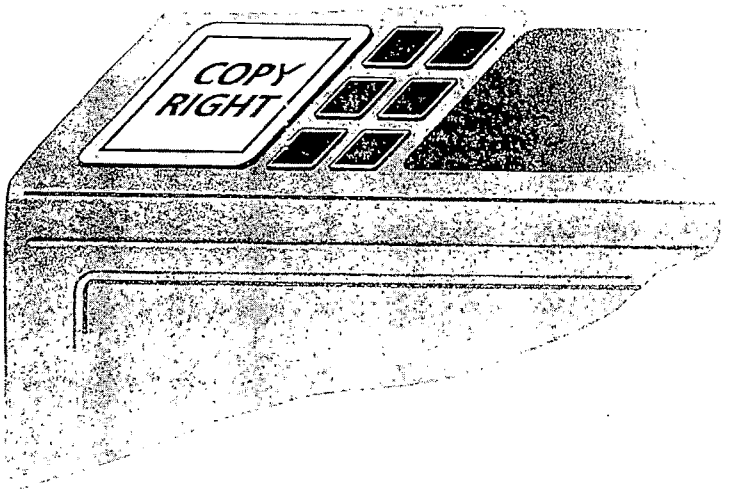
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CLINICAL SECTION

Clinical Opinion

Selection bias in in vitro fertilization programs

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New Haven, Connecticut

Pregnancy rates per cycle reported from different in vitro fertilization–embryo transfer programs vary widely. While several programs have reported constant pregnancy rates per cycle, others report declining pregnancy rates. Selection biases at the point of entry and between cycles are discussed as possible explanations of these discrepancies. (AM J OBSTET GYNECOL 1992;166:1-3.)

Key words: In vitro fertilization, pregnancies, pregnancy rate, selection

In vitro fertilization–embryo transfer pregnancy rates have been used to monitor in vitro fertilization program success rates and to measure the impact of the assisted reproductive technologies on public health.[1] A virtual lack of standardization in reporting has created substantial confusion in the field, rendering assessment of individual programs difficult and prompting accusations of distortion and abuse.[2] With no acknowledged measure for success, the true differences that exist between individual in vitro fertilization–embryo transfer centers have been clouded. This situation had led to a call for increased supervision of in vitro fertilization clinics and the reporting of clinic-specific statistics.[3] Indeed, results from in vitro fertilization-embryo transfer programs in the United States have been available for the last 3 years through the in vitro fertilization-embryo transfer registry,[4-6] which in 1988, the last year reported, included 135 clinics.[6] Although the registry did not contain clinic-specific data, it provided an acceptable reporting format and mean pregnancy rates from the reporting clinics.

The pregnancy rate continues to be used as the criterion of program success. As shown in Table I, several programs[7-9] have reported nearly constant pregnancy rates per cycle, suggestive of a homogeneous patient population where pregnancy ultimately occurs for those who are able to undergo a sufficient number of trials. By contrast, data from Yale University[10] and other reports[11] reflect declining pregnancy rates per cycle, suggesting a heterogeneous patient population in which patients have various probabilities of success in a given cycle.

Statistical and mathematical analyses may be used to formalize the patterns noted here. For example, Padilla and Garcia[9] and Bustillo et al.[8] used life-table analysis to estimate pregnancy rates per cycle, whereas Guzick et al.[7] and Kaplan et al.[10] presented mathematical models that explicitly characterized heterogeneity of various forms. In both the statistical and modeling approaches, women who discontinue treatment without achieving pregnancy are assumed to drop out independently of the treatment process. This fallacy underlies the statistical methods of essentially all studies to date on the performance of in vitro fertilization programs: Reasonable assurance must exist that the patients who drop out of treatment do not differ significantly from those remaining in treatment from cycle to cycle.

A closer look at the literature raises some real concerns. It appears that almost all of the pregnancies achieved in the available series occur during the first four attempts. Specifically, of all women who become pregnant, the percentages achieved within four cycles at Norfolk, Baltimore, Fairfax, and Yale are 93%, 94%, 93%, and 98%, respectively.[7-10] However, whereas the pregnancy rate per cycle continues unchanged beyond the fourth cycle in the other three programs, this is not the case at Yale (Table I). In what ways might these programs truly differ?

Couples undergoing in vitro fertilization represent a subpopulation of all infertile couples. This subpopulation is formed by the application of nonstandard selection criteria. There are several branch points on the

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Table I. Pregnancy rate per cycle in different in vitro fertilization–embryo transfer programs (percent)

	Cycle No.									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Norfolk[7] (per retrieval)	13.6	13.0	17.5	17.0	8.3	15.4	37.5	0	100	—
Yale[10] (per retrieval)	13.1	10.7	6.9	4.3	4.9	0	0	0	0	
Fairfax[8] (per retrieval)	15	13.8	10.2	9	11.8	6	6.4	4.7	16.6	0
Baltimore[9] (per transfer)	25	29	28	33	35	30	40	—	—	—

in vitro fertilization–embryo transfer dendrogram, which may alter the composition of the resulting patient population. Combined with the nonrandom dropout as discussed above, these two selection modalities confound the interpretation of pregnancy rate outcome in in vitro fertilization programs.

Reduced fertility potential is a broad spectrum of conditions, ranging from mild subfertility, where unassisted conception takes somewhat longer than 1 year, to severe infertility, where conception does not occur after many years, in spite of multiple therapeutic attempts, such as controlled ovarian hyperstimulation, artificial insemination, and in vitro fertilization–embryo transfer. The potential patient population is thus composed of patients who are distributed along a clinical continuum. The distinct infertility subpopulation that is selected for in vitro fertilization–embryo transfer will significantly affect the observed success rate.

Clearly, programs that limit the age of patients to <40 years eliminate those with decreased fecundity On the other hand, more liberal entry criteria, for example, at Yale, have allowed women up to 45 years old to participate in the program. In addition, some programs consider in vitro fertilization–embryo transfer an 'end stage" treatment, where only patients with prolonged infertility who have gone through all other reasonable treatments and have not conceived are included. Other programs tend to use in vitro fertilization–embryo transfer much earlier. Recently, Gindoff et al.[12] have reported oocyte retrieval and transfer during a diagnostic laparoscopy for infertility. It is clear that a certain proportion of patients, if they had not been treated by in vitro fertilization–embryo transfer at an early point in the infertility workup, would have conceived with expectant management, whereas additional pregnancies would have resulted from other less agressive, non–in vitro fertilization treatments. Inclusion of these "more fertile" patients would thus increase the reportable pregnancy rates.

Selection bias does not stop at the point of entry to in vitro fertilization–embryo transfer but continues between cycles. The dropout population is determined largely by physicians, either directly or indirectly, by shaping patient decisions via counseling.

If a program is very strict in "dropping" patients who have had poor response to controlled ovarian hyperstimulation or in whom fertilization did not occur, the ongoing population in the next cycle should have a higher conception rate than would occur in a program that allows such patients to continue.

Among in vitro fertilization–embryo transfer programs across the country there is a wide variety of selection biases of the sorts described. Programs that select more fertile patients will have more homogeneous-appearing pregnancy data, especially if patients of lesser or no potential to conceive continue to be "extracted." Programs that include the more infertile patients will have more heterogeneous-appearing conception patterns, owing to the presence of the more diverse population, that is, an admixture of both fertile and infertile women.

In summary, two sources of selection bias plague in vitro fertilization–embryo transfer programs. Different programs enforce different eligibility criteria creating differential patient selection across programs at intake. Also, some programs may encourage patients to discontinue in vitro fertilization–embryo transfer on the basis of poor performance in previous cycles whereas other programs experience random dropout during treatment. The implication of these biases is that pregnancy rates reported by different in vitro fertilization–embryo transfer programs do not provide a basis for fair comparison. A method for the standardization of in vitro fertilization–embryo transfer programs would involve the characterization of patients at intake to the in vitro fertilization program and the characterization of those patients who drop out between cycles, in addition to studying those women who ultimately achieve viable pregnancies.

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Main clinical types and subtypes of eclampsia

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Eclampsia has been traditionally divided in three types: antepartum, intrapartum, and postpartum. Several authors consider two more subtypes, early cases and intercurrent eclampsia. The clinical analysis of 990 patients with eclampsia divided according to such classification revealed numerous significant differences that could give grounds for the interpretation of conflicting results in medical research. Maternal and perinatal mortality, types and incidence of complications, obstetric and eclamptic profiles, and incidence of underlying diseases were strikingly higher in antepartum eclampsia, especially in early cases. The features of intrapartum eclampsia were closer to those of the postpartum group than to those of antepartum cases, and intercurrent eclampsia was oddly benign for the mother but not for the fetuses. These findings indicate that a more precise classification of eclampsia must consider seven differential facts: (1) timing of convulsions, (2) length of pregnancy, (3) complications, (4) underlying diseases, (5) maternal age, (6) number of index pregnancy, and (7) single or multiple gestation. (Am J Obstet Gynecol 1992;166:4-9.)

Key words: Types of eclampsia, maternal mortality, perinatal mortality

Efforts to understand the hypertensive complications of pregnancy have been directed mainly to the investigation of their pathologic characteristics, early diagnosis, and treatment. The subject of their definition, classification, and nomenclature remains unsettled, although several authors, institutions, and related medical societies have issued recommendations for local and international acceptance.[1-7] Furthermore, most recent publications on this matter focus their interest on the nonconvulsive stages of the disease and seem to leave the problem of eclampsia practically untouched.[6,7]

We thought that, to clarify many important questions and provide a better basis for the interpretation of conflicting results, it was necessary to revise the conventional classification of patients with eclampsia with study of the most important clinical characteristics of each type, to look for the differences that could improve such discrimination. This objective appeared to be worthwhile because eclampsia still is the leading cause of maternal death and perinatal morbidity in nonprivileged populations and will continue to be studied intensively throughout the world. In addition, some of the findings of this study could become useful for the understanding of other obscure aspects of the lesser degrees of this condition.

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Material and methods

All patients admitted to the Hospital de Gineco-Obstetricia No. 2 del Centro Médico Nacional del I.M.S.S. in Mexico City with a well-documented diagnosis of eclampsia from July 1, 1963, to Sept. 19, 1985, were included in the study. These patients came from the heterogeneous population of one of the largest cities in the world, but the overwhelming majority belonged to the economically disadvantaged and, for various reasons, they represented the advanced stages of the disease.

Definitions. Three main clinical types and two subtypes of eclampsia were considered, as defined here. Antepartum eclampsia was defined as generalized convulsions starting clearly before the beginning of labor in a pregnant woman with evident hypertension or proteinuria; intrapartum eclampsia included convulsions starting after the beginning of labor in a pregnant woman with evident hypertension or proteinuria; postpartum eclampsia was defined as convulsions appearing within 7 days after delivery of the fetus and the placenta in a patient with hypertension or proteinuria during pregnancy, labor, or the postdelivery period. Early antepartum eclampsia consisted of convulsions, hypertension, and proteinuria appearing ≤28 weeks' gestation, and intercurrent eclampsia consisted of convulsions, hypertension, or proteinuria appearing as in antepartum cases but stopping and subsiding with enough clinical improvement to allow continuation of pregnancy for >7 days. Since generalized convulsions were the leading symptom, all noneclamptic causes

Table I. Maternal mortality and complications in the three main clinical types of eclampsia

	Intrapartum (%)	*Antepartum* (%)	*Postpartum* (%)
No. of cases	187	620	160
Maternal mortality			
Total	10.2*	16.8*	9.4
Corrected†	8.2	9.9	5.8
Brain hemorrhage	8.0	12.3	7.5
Minor cerebrovascular accident	6.4	3.4	4.4
Disseminated intravascular coagulation	7.5*	14.0	11.9
Acute renal failure	6.4*	11.9	7.5
Abruptio placentae	3.7*	7.9	6.3
Postpartum hemorrhage	8.0	5.5*	10.6
Cardiac arrest	4.3	6.1	5.6
Chronic respiratory failure	3.2	3.6	3.1
Bronchial aspiration	1.1	2.7	1.3
Acute pulmonary edema	2.2	2.3	0.6
Ruptured liver	0	1.3	1.3
TOTAL	90‡	440*	96*

Footnote symbol at extreme right indicates significance between first and last column of data.
*$p < 0.05$.
†After exclusion of patients who were moribund on admission.
‡$p < 0.001$.

were thoroughly ruled out. Cases of eclampsia in multiple pregnancy were not included, because they have been already reported.[8]

Methods. The clinical variables for analysis and comparison were maternal and perinatal mortality, incidence of complications, incidence of cesarean operation, obstetric profile (maternal age, parity, amenorrhea, and fetal birth weight), eclamptic symptoms (systolic and diastolic hypertension, average proteinuria, total number of convulsions, blood urea and creatinine, and average number of platelets), and presence of underlying diseases. The treatment given to these patients has been outlined in a previous publication,[9] and it is not a matter for analysis here.

Statistics. Proportions are presented as percentages. All mean values are presented as ±1 SE of the mean. The paired t test, χ^2 test, and Fisher's exact test were used to estimate the statistical significance of the differences. Three p values are reported: <0.05, <0.01, and <0.001.

Analysis of the data

Cases. A total of 990 patients were studied: 620 with antepartum and 187 with intrapartum eclampsia, 160 with the postpartum type, and 23 with the intercurrent variety. There were 54 cases in the antepartum eclampsia group with onset at or before the twenty-eighth week.

Maternal mortality and complications. When total maternal mortality was considered, the highest rate was found in the early eclampsia cases (22.2%) and the lowest one in the intercurrent eclampsia subtype (0%). Significant differences existed between antepartum eclampsia and either intrapartum or postpartum cases (Tables I and II). This significance disappeared when the figures were corrected by the exclusion of moribund patients on admission to the hospital.

As far as the frequency of complications was concerned, the highest incidence of brain hemorrhage belonged to early eclampsia (24.1%) and the lowest one to intercurrent eclampsia (4.3%). The figures for disseminated intravascular coagulation, acute renal failure, and abruptio placentae were significantly higher in antepartum eclampsia in comparison with intrapartum cases, and the frequency of severe postpartum hemorrhage was significantly higher in postpartum eclampsia (10.6%) in comparison with antepartum cases (5.5%). There were no significant differences in the incidence of other less frequent complications (cardiac arrest, bronchial aspiration, acute pulmonary edema, progressive respiratory failure, and liver rupture). Nevertheless, when all complications are considered, it becomes obvious that antepartum eclampsia, especially early eclampsia, carried the worse effects.

Perinatal mortality. Total perinatal mortality was highest in early eclampsia (92.6%) followed by intercurrent cases (47.8%) and antepartum eclampsia (31.4%), and it was significantly lower in intrapartum and postpartum cases (12.8% and 7.9%, respectively). These significant differences persisted for fetuses weighing >1000 gm (Tables III and IV). The highest rates of total fetal mortality were found in the early eclampsia subtype (33.3%) and in antepartum eclampsia (18.3%). Total neonatal mortality was highest in both

Table II. Maternal mortality and complications in two clinical subtypes of antepartum eclampsia

	Early eclampsia (%)	*Antepartum (%)*	*Intercurrent (%)*
No. of cases	54	620	23
Maternal mortality			
Total	22.2	16.8*	0†
Corrected†	12.5	9.9	0
Brain hemorrhage	24.1*	12.3	4.3*
Minor cerebrovascular accident	3.7	3.4	4.3
Disseminated intravascular coagulation	18.5	14.0	4.3
Acute renal failure	13.0	11.9	8.7
Abruptio placentae	5.6	7.9	0
Postpartum hemorrhage	3.7	5.5	0
Cardiac arrest	5.6	6.1	0
Chronic respiratory failure	11.1	3.6	0
Bronchial aspiration	1.8	2.7	0
Acute pulmonary edema	0	2.3	0
Ruptured liver	1.8	1.3	0
TOTAL	48†	440§	5§

Footnote symbol at extreme right indicates significance between first and last column of data.
*$p < 0.05$.
†$p < 0.01$.
‡After exclusion of patients who were moribund on admission.
§$p < 0.001$.

Table III. Perinatal mortality and incidence of cesarean operation in the three main clinical types of eclampsia

	Intrapartum (%)	*Antepartum (%)*	*Postpartum (%)*
Perinatal mortality			
Total	12.8*	31.4*	7.9
>1000 gm	12.4*	26.2*	4.8†
Fetal mortality			
Total	10.7†	18.3*	4.0†
>1000 gm	10.2	16.3*	2.7†
Neonatal mortality			
Total	2.4*	15.7*	4.1
>1000 gm	2.4‡	10.6‡	2.1
Incidence of cesarean operation	27.8*	74.3*	37.7

Footnote symbol at extreme right indicates significance between first and last column of data.
*$p < 0.001$.
†$p < 0.05$.
‡$p < 0.01$.

subtypes of antepartum eclampsia (early eclampsia, 59.3%; intercurrent, 25%), and the lowest total and corrected figures belonged to intrapartum and postpartum eclampsia (2.4 and 4.1, total; 2.4 and 2.1% corrected figures, respectively). The highest incidence of cesarean operation belonged to antepartum eclampsia (74.3%), it dropped to 51.8% for the early eclampsia subtype, and it fluctuated between 28% and 38% in the remaining groups.

Main clinical profile. There were no significant differences in the ages of the mothers in the three main types of eclampsia. However, maternal age was significantly higher in both subtypes of antepartum eclampsia (27.1 years for early eclampsia and 27.9 for intercurrent cases). Obviously, the most significant differences were found in the length of pregnancy and fetal birth weight in all types and subtypes of patients. The lowest average fetal weight belonged to early eclampsia (943 gm), followed by intercurrent cases (1515 gm), and the highest figures were found in intrapartum and postpartum eclampsia cases (2821 and 2711 gm, respectively) (Tables V and VI).

Systolic and diastolic hypertension and proteinuria were highest in antepartum eclampsia, especially in the early cases. Similarly, plasma urea and blood creatinine levels were highest in this subgroup of patients (Tables V and VI). The figures for total blood hemoglobin and plasma proteins of the three main groups also showed significant differences, but they may reflect more the physiologic or therapeutic blood volume changes as-

Table IV. Perinatal mortality and incidence of cesarean operation in two clinical subtypes of antepartum eclampsia

	Early eclampsia (%)	*Antepartum* (%)	*Intercurrent* (%)
Perinatal mortality			
Total	92.6*	31.4	47.8*
>1000 gm	71.4*	26.2	33.3†
Fetal mortality			
Total	33.3†	18.3	30.5
>1000 gm	21.4	16.3	20.0
Neonatal mortality			
Total	59.3*	15.7	25.0†
>1000 gm	63.6*	10.6	14.3†
Incidence of cesarean operation	51.8*	74.3*	34.8

Footnote symbol at extreme right indicates significance between first and last column of data.
*$p < 0.001$.
†$p < 0.05$.

Table V. Clinical data in three main types of eclampsia

	Intrapartum	*Antepartum*	*Postpartum*
No. of cases	187	620	160
Maternal age (yr)	23.7 ± 0.49	24.7 ± 0.27	25.1 ± 0.50*
Pregnancies (No.)	2.45 ± 0.19	2.80 ± 0.11	3.05 ± 0.22*
Gestational age (wk)	38.8 ± 0.17†	35.0 ± 0.17†	38 ± 0.26*
Fetal weight (gm)	2,821 ± 49†	2,117 ± 31†	2,711 ± 60
Systolic blood pressure (mm Hg)	168 ± 1.68†	175 ± 0.95†	168 ± 1.96
Diastolic blood pressure (mm Hg)	113 ± 0.94†	120 ± 0.61†	114 ± 1.22
Proteinuria (gm/L)	4.61 ± 0.5†	7.3 ± 0.41†	4 ± 0.37
Total No. of seizures	3.92 ± 0.35	4.1 ± 0.14*	3.35 ± 0.33
Blood hemoglobin (gm/dl)	11.3 ± 0.16†	12.3 ± 0.08*	11.9 ± 0.17‡
Plasma proteins (gm/dl)	4.7 ± 0.05†	4.92 ± 0.03	4.95 ± 0.06‡
Plasma urea (mg/dl)	31.6 ± 1.56*	36.2 ± 1.16	32 ± 1.87
Blood creatinine (mg/dl)	1.3 ± 0.06	1.4 ± 0.04	1.34 ± 0.09
Platelet count (No./mm³)	153,000 ± 5,308†	125,899 ± 2,860†	90,355 ± 7,143†

Values are mean ± SE. Footnote symbol at extreme right indicates significance between first and last column of data. All clinical and laboratory data were gathered as closely as possible to the critical period. Blood pressure figures represent averages of this period.
*$p < 0.05$.
†$p < 0.001$.
‡$p < 0.01$.

sociated with labor and delivery than a direct eclamptic feature. The platelet count was significantly lower in postpartum eclampsia (90,355/mm³). However, this was probably related to the high incidence of postpartum hemorrhage (Table I), in association with various obstetric complications that make it not a true eclamptic thrombocytopenia, as occurred in early eclampsia cases (117,956/mm³), where the incidence of postpartum hemorrhage was only 3.7%, in spite of the highest incidence of disseminated intravascular coagulation (18.5%). Finally, although early eclampsia and antepartum eclampsia patients had more convulsive seizures, the differences were not statistically significant.

Previous underlying diseases. To establish the presence of chronic hypertension, diabetes mellitus, renal disease, or cardiac disease, all patients were followed up for at least 6 months after delivery, and a large majority were followed up through their next pregnancies. The combined incidences of these diagnoses in the three main types of eclampsia were 17.9%, 13.4%, and 16.3% for antepartum, intrapartum, and postpartum eclampsia, respectively. The differences were not significant. However, the figures for early eclampsia and for intercurrent cases were 42.6% and 52.2%, respectively ($p < 0.001$ in comparison with the main antepartum type). Chronic hypertension was by far the commonest diagnosis, because it constituted >50% of all underlying diseases in every group. Again, the incidence of chronic hypertension in early eclampsia and in intercurrent cases was higher (29.6% and 26.1%, respectively); however, because of the small number of patients in the intercurrent subtype, the only

Table VI. Main clinical data in two subtypes of antepartum eclampsia

	Early eclampsia	*Antepartum*	*Intercurrent*
No. of cases	54	620	23
Maternal age (yr)	27.1 ± 0.85*	24.7 ± 0.27†	27.9 ± 1.46
Pregnancies (No.)	3.4 ± 0.38	2.8 ± 0.11	4.1 ± 0.67
Gestational age (wk)	26 ± 0.23‡	35 ± 0.17‡	31.7 ± 0.79‡
Fetal weight (gm)	943 ± 46‡	2,117 ± 31‡	1,515 ± 162‡
Systolic blood pressure (mm Hg)	183 ± 3.1†	175 ± 0.95*	163 ± 3.7‡
Diastolic blood pressure (mm Hg)	124 ± 2†	120 ± 0.61*	113 ± 2.1‡
Proteinuria (gm/L)	6.74 ± 1.1	7.3 ± 0.41‡	3.76 ± 0.8†
Total No. of seizures	4.4 ± 0.6	4.1 ± 0.14	3.1 ± 0.6
Blood hemoglobin (gm/dl)	12.2 ± 0.3	12.3 ± 0.08	12 ± 0.36
Plasma proteins (gm/dl)	5 ± 0.09	4.92 ± 0.03	5.1 ± 0.14
Plasma urea (mg/dl)	51.3 ± 8.8†	36.2 ± 1.16	33.7 ± 3.6
Blood creatinine (mg/dl)	2 ± 0.3†	1.4 ± 0.04*	1.07 ± 0.1*
Platelet count/mm³	117,956 ± 9,122	125,899 ± 2,860	137,500 ± 23,039

Values are mean ± SE. Footnote symbol at extreme right indicates significance between first and last column of data. All clinical and laboratory data were gathered as closely as possible to the critical period. Blood pressure figures represent averages of this period.

*$p < 0.01$.

†$p < 0.05$.

‡$p < 0.001$.

significant difference was between early cases and antepartum eclampsia proper ($p < 0.001$).

Comment

The groups of eclamptic patients in this report were as clearly defined as possible. Hence, their clinical characteristics must be considered representative of their respective categories. However, because the precise diagnosis of the onset of labor is not easily ascertainable and eclampsia per se increases uterine contractility and leads to premature labor, a discrete overlap between antepartum and intrapartum cases may exist. Nevertheless, our data showed that intrapartum eclampsia was closer to the postpartum group than to any of the others.

The most influential factor marking the severity of the disease was the timing of the critical process. Obviously, this was a determinant of all perinatal variables, but it was also associated with important maternal data, except in the small group of intercurrent eclampsia cases, where the findings showed that this disease occasionally may be strangely benign for the mother.

The incidence of underlying diseases and the types and rates of maternal complications in the various groups suggest that the main reason for the severity of eclampsia and for the early or late onset of the problem is, in the large majority of the cases, this previous pathologic condition. However, because the severity of any disease is not usually caused by a single factor but by a combination of very diverse conditions and circumstances, there were large numbers of patients apparently free of underlying disease, even among the earliest and most severe of the cases. Many of the early eclampsia patients and an important number of the women with the worst cases of antepartum eclampsia opted for definitive contraception, and the reallocation of the entire obstetric population that followed the destruction of our institution at the end of 1985 made long-term evaluations extremely difficult. We suspect that many of those women apparently free of underlying disease will show the signs of chronic hypertension in the course of time. It is evident that many diseases will not show all their characteristics until a certain number of cases are studied. If the incidence is rather low, this fact may become an insurmountable problem. The solution offered by collaborative studies has several disadvantages. The most important ones depend on differences in the populations and in the medical criteria, but in many instances they become the best possibility.

The material included in this report belonged to a single institution, thus avoiding some of the limitations of collaborative studies, but it was gathered through a span of 22 years, which may be considered a weakness. However, the fact that these patients were handled practically by the same medical and paramedical staff throughout that period may compensate for it.

One of the limitations of studying diseases through relatively long time lapses is that the morbidity data may show continuous changes as a result of technologic and basic health improvements that make the last cases quite different from the early ones. However, in our material, morbidity was practically constant through the 22 years, and if there was a change, it was toward an increase in the incidence and severity of the problem in the last years.

The data presented in this report, added to those of our former partial observations,[9-12] clearly indicate that a complete and precise classification of eclampsia cannot be based on a single aspect of the disease. At the least, it must account for the following differential facts: (1) timing of convulsions in regard to labor and delivery, (2) length of pregnancy at onset of the condition, (3) presence of significant semispecific complications, (4) presence of underlying diseases, (5) age of the patient, (6) gravidity, and (7) single or multiple gestation. As long as no such classification exists, the results of many investigations on eclampsia will be more conflicting than enlightening. We hope that our study belongs more to the second than to the first category.

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Prognosis in fetal diaphragmatic hernia

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The reported mortality for prenatally detected congenital diaphragmatic hernia is high. Polyhydramnios and presentation in early pregnancy have been suggested as high-risk factors adversely affecting outcome. We retrospectively reviewed 55 cases of congenital diaphragmatic hernia diagnosed prenatally in our unit. There was an overall mortality of 73%. The mortality in cases with presentation before 25 weeks' gestation was 74%, if the cases resulting in termination of pregnancy are excluded, compared with a mortality of 60% in those seen after this gestational age. Underdevelopment of left-sided cardiac structures was found to be a helpful prognostic factor. We were unable to confirm the predictive nature of hydramnios. Associated chromosomal anomalies were found in two fetuses and major congenital heart disease in nine. Although diagnosis before 25 weeks' gestation is associated with a higher mortality than in cases detected later, it is not universally fatal. If congenital heart disease and chromosomal anomalies are excluded and there is little or no evidence of left heart underdevelopment, the odds for survival will improve. This should be taken into account when the management of these cases is planned. (AM J OBSTET GYNECOL 1992;166:9-13.)

Key words: Left heart underdevelopment, fetal diaphragmatic hernia

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Congenital diaphragmatic hernia has been variously reported as occurring in 1 in 2000 to 1 in 5000 births.[1, 2] The prenatal diagnosis of congenital diaphragmatic hernia is well established,[3] but the outcome related to antenatal detection has been poor.[4, 5] In a

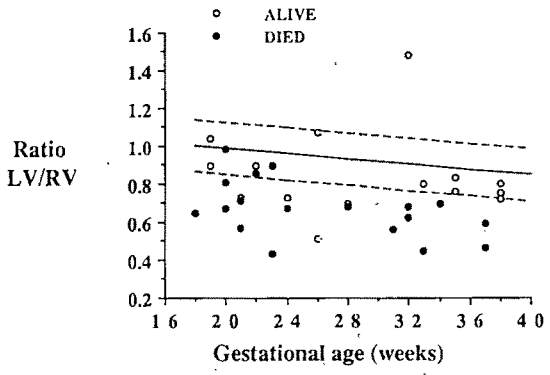


Fig. 1. Scatter of values of ratio of left-to-right ventricular size *(LV/RV)* in 33 cases, compared with normal range for gestational age. For normal range *solid line* is mean and *dotted lines* are 95% confidence intervals.

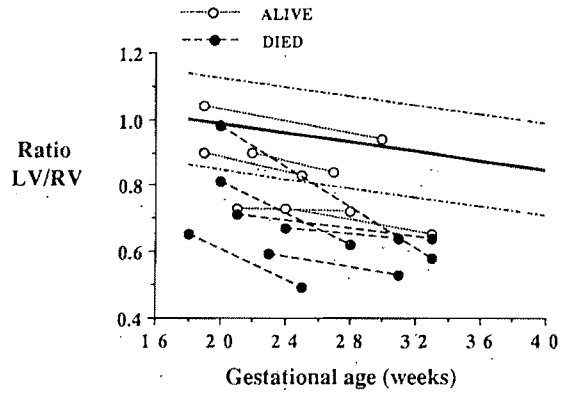


Fig. 2. Sequential values of ratio of left-to-right ventricular *(LV/RV)* size in 11 cases, compared with normal range for gestational age. For normal range *solid line* is mean and *dotted lines* are 95% confidence intervals.

recent report of prenatally diagnosed diaphragmatic hernia[6] there were no survivors of 14 cases seen before 25 weeks' gestation, in spite of intensive neonatal care, including the use of extracorporeal membrane oxygenation. The authors suggested that early diagnosis per se was an important factor influencing the outcome. Polyhydramnios was found to be an additional indicator of a poor prognosis. Its presence was associated with an 82% mortality rate. This experience of the natural history has been used to justify prenatal operative techniques involving hysterotomy.[7] However, as the resource implications of both extracorporeal membrane oxygenation and prenatal operation are very great, it is important to reevaluate the outcome in this condition. We therefore retrospectively examined 55 cases of fetal congenital diaphragmatic hernia seen in our department over a 10-year period. Factors that might help to predict the prognosis for cases detected prenatally were sought, so that a rational protocol for future management of pregnancy could be formulated.

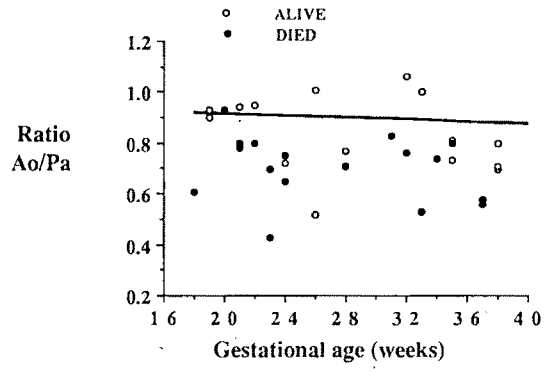


Fig. 3. Scatter of values of ratio of aorta to pulmonary artery *(Ao/Pa)* in 32 cases, compared with normal mean for gestational age.

Patients and methods

Over a 10-year period 55 fetuses with a diaphragmatic hernia have been evaluated by fetal echocardiography in our unit. The diagnosis of fetal anomalies by ultrasonography has been approved by the ethics committee, and each mother gave informed consent to the study. The presence of congenital diaphragmatic hernia found at a routine obstetric scan was the reason for referral in 44 cases. In nine cases a cardiac abnormality had been suspected, and one case was referred after the detection of a pleural effusion. The remaining case was referred for fetal echocardiography because the parents had had a previous child with congenital heart disease.

Each fetus was examined with either a Hewlett Packard 77020 phased array system or an Advanced Technology Laboratories Ultramark 4 system. This was a retrospective study, and a videotape recording of each examination was reviewed. The diagnosis of congenital diaphragmatic hernia was made if there was displacement of the fetal heart associated with an intrathoracic mass having the appearance of stomach or liver. Ultrasonographic diagnosis of polyhydramnios was made when the four quadrant measurements of amniotic fluid were >16 cm.

The structure of the fetal heart was examined for evidence of associated congenital heart disease. The maximum widths of the left and right ventricles were measured in a four-chamber projection, and the ratio of the left ventricle to the right ventricle was compared with the normal range for gestational age.[8] The diameters of the aortic root and the pulmonary valve orifice were measured, and the ratio of aorta to pulmonary artery was compared with the normal mean for gestational age.[9] In 12 fetuses sequential studies were performed during pregnancy. It was possible to sequentially measure the left ventricle/right ventricle ratio in 11 of these, and the aorta/pulmonary artery ratios in nine. Measurements of the cardiac structures

Table I. Associated anomalies

Type of abnormality	*Gestational age (wk)*	*Chromosomal anomaly*	*Outcome*
Cardiac			
Ventricular septal defect	31	Trisomy 18	Intrauterine death
Ventricular septal defect	33	No	Neonatal death
Ventricular septal defect	21	No	Termination of pregnancy
Atrioventricular septal defect	37	Deletion 8	Neonatal death
Atrioventricular septal defect	33	No	Neonatal death
Hypoplastic left heart syndrome	20	No	Termination of pregnancy
Hypoplastic left heart syndrome	22	No	Neonatal death
Tetralogy of Fallot	18	No	Termination of pregnancy
Pulmonary atresia, ventricular septal defect	18	No	Termination of pregnancy
Noncardiac			
Absent kidney, cleft palate	31	No	Intrauterine contraceptive device
Cleft palate	35	No	Alive
Pleural effusion	26	No	Alive
Pleural effusion	37	No	Alive

were performed only in the 39 cases where the pregnancy continued and where there was no evidence of congenital heart disease, so that these could be related to outcome. Images adequate for satisfactory measurements were not recorded on the available videotape in all cases. However, it was possible to obtain a left ventricle/right ventricle ratio in 33 of 39 cases (85%) and the aorta/pulmonary artery ratio in 32 of 39 (82%).

The outcome of each pregnancy was noted and related to the gestational age at the time of presentation. Evidence of associated anomalies, both at the time of ultrasonographic examination and on subsequent examination, was noted. In all cases the diagnosis of diaphragmatic hernia was confirmed either postnatally or at autopsy.

Results

The diaphragmatic defect was left-sided in 53 cases, right-sided in one, and retrosternal in one.

Polyhydramnios. Polyhydramnios was a feature in 16 of the 55 cases (29%). Termination of pregnancy took place in four of the 16, one case resulted in a spontaneous intrauterine death, five of the babies died postnatally, and there are six survivors, three of whom were seen before 25 weeks of pregnancy. Congenital heart disease was present in three cases associated with polyhydramnios.

Ratio of left-to-right ventricular size. The left ventricle/right ventricle ratio obtained in 33 cases is shown in Fig. 1. The majority of survivors (73%) had a left/right ventricular ratio within the normal range, whereas the majority of nonsurvivors (83%) had a reduced ratio. The left ventricle/right ventricle ratio of the survivors (mean ± SD 0.848, ±0.233) was significantly different ($p < 0.01$) from that of the nonsurvivors (mean ± SD 0.666 ± 0.151). In the one case with a high ratio, the diaphragmatic hernia was right-sided and the heart was pushed into the left chest. In this fetus the right-sided heart structures appeared smaller than the left. Sequential observations of the left ventricle/right ventricle ratio were obtained in 11 fetuses (Fig. 2). In three of these the sequential ratio was markedly reduced, and none of the three survived.

Ratio of aorta to pulmonary artery. The aorta/pulmonary artery ratio was obtained in 32 fetuses (Fig. 3). The ratio of the survivors (mean ± SD 0.837 0.149) was significantly different ($p < 0.05$) from that of the nonsurvivors (mean ± SD 0.704 0.128). A sequential value was obtained in nine fetuses, and the changes with gestational age are shown in Fig. 4.

Outcome. The outcome of the 55 cases of diaphragmatic hernia detected prenatally is shown in Fig. 5. The parents elected termination of pregnancy in 11 cases. Of the 44 continuing pregnancies, three resulted in a spontaneous intrauterine death, and 23 babies died in the neonatal period and three in infancy. There are currently 15 survivors, 33% of whom were seen before 25 weeks' gestation and 67% after this gestational age. Two children are <6 months of age, three have survived beyond 2 years, four beyond 3 years, and six beyond 4 years. Of the survivors, one child has had problems with esophageal reflux, one has had problems related to a cleft palate, and one child has scoliosis. There have been no long-term problems in the remaining. Our policy has been to deliver these babies vaginally whenever possible. Of the total group, seven were delivered by cesarean section, four of whom are survivors. In 28 cases antenatal care was transferred for delivery to take place in a unit with specialized pediatric surgical facilities available. In 12 of the 15 survivors delivery took place in a specialized unit.

Associated anomalies. An associated anomaly was present in 13 cases (23.6%). Congenital heart disease was the most frequent associated lesion occurring in nine of these 13 cases (16.4% of the total group). A chromosomal anomaly occurred in two cases (3.6%),

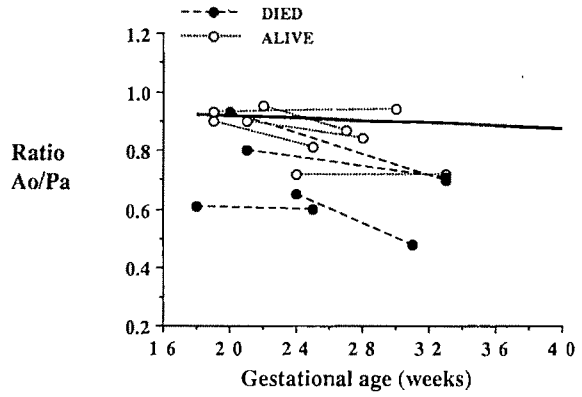


Fig. 4. Sequential values of ratio of aorta to pulmonary artery *(Ao/Pa)* in nine cases, compared with normal mean for gestational age.

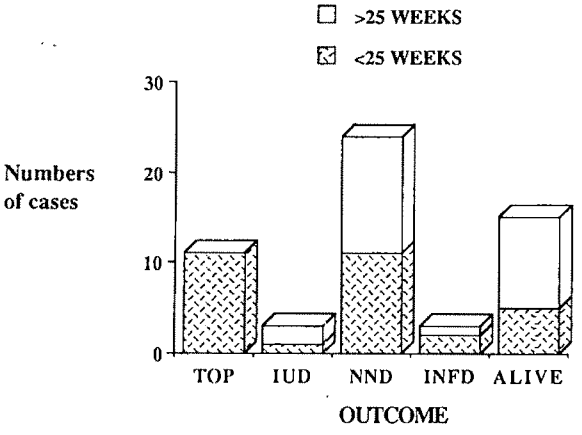


Fig. 5. Outcome in 55 cases of fetal diaphragmatic hernia in cases with presentation before 25 weeks of gestation, compared with cases with presentation after 25 weeks.

both of whom also had congenital heart disease. A breakdown of the different types of anomaly is shown in Table I.

Comment

The mortality associated with congenital diaphragmatic hernia detected prenatally is higher than that reported postnatally, because the spectrum of disease seen in the fetus is different.[2] Several reports have suggested that death occurs in >75% of cases of congenital diaphragmatic hernia that are detected before birth in spite of optimum postnatal care.[4, 6] The overall mortality in our series was 73%, but it fell to 66% if cases that elected termination of pregnancy were excluded and to 62% if those associated with congenital heart disease were also excluded. In cases with presentation before 25 weeks' gestation (n = 30) the overall mortality was 83% but fell to 72% if cases resulting in a termination or those associated with congenital heart disease were excluded. In the group seen after 25 weeks' gestation (n = 25), the overall mortality was 60% and fell to 52% if the four cases that also had congenital heart disease were excluded. Thus, although the mortality associated with the cases in our series was high, it was better than that previously reported.

We were unable to confirm previous reports that polyhydramnios is a predictor of a poor outcome.[6, 7] Hydramnios was a feature of 16 cases (29%), 12 of which had an isolated hernia. Of these 12 cases, termination of pregnancy took place in two and there are six survivors. It has been suggested that the presence of stomach in the chest, especially if it is dilated, may also be predictive of a poor outcome.[7] We were unable to relate this finding to outcome, because in six of our 15 survivors a dilated stomach was identified in the chest.

Left heart underdevelopment has previously been reported as being associated with a poor outcome and cardiac ventricular disproportion seen before 24 weeks was associated with a 100% mortality.[10] Our study confirmed the finding that disproportion, as shown by a reduced left-to-right ventricular size, is predictive of a poor outcome. However, two of our survivors had marked ventricular disproportion, one at 21 weeks' gestation and the other at 24 weeks' gestation, so that although a reduced left-to-right ventricular ratio is likely to be associated with a poor outcome, it is not an absolute prognostic indicator. In addition, three of the cases seen in early pregnancy with a left-to-right ventricular ratio in the normal range did not survive. Sequential observations, however, showed that changes that will alter the prognosis can occur with advancing gestational age. In three of the cases with sequential studies, the left ventricle/right ventricle ratio fell much more dramatically than expected with advancing gestational age, indicating more marked left ventricular underdevelopment. None of the three babies survived. Progression of lesions has been reported with some types of congenital heart disease[11] and emphasizes the need for serial scans during pregnancy for reassessment. Similarly, the ratio of the aorta to the pulmonary artery was generally lower than the mean in the nonsurvivors compared with the survivors, and changes in this ratio were also observed with advancing gestation. Thus examination of the fetal heart may be helpful in distinguishing cases likely to be associated with a good or bad outcome.

The cause of left heart underdevelopment remains uncertain. It has been suggested that herniated organs cause compression of the left ventricle in left-sided diaphragmatic hernias.[12] The development of cardiac chambers is related to the volume of blood flow, so that reduced blood flow will result in underdevelopment. In the presence of a left-sided diaphragmatic hernia, it is likely that reduced blood flow into the left side of

the heart is due to a combination of increased left atrial pressure restricting the normal right-to-left interatrial flow and decreased pulmonary venous flow because of lung hypoplasia. However, the evidence of right heart underdevelopment in the right-sided hernia suggests that direct compression of cardiac structures may play a role in hypoplasia.

The association of congenital heart disease with congenital diaphragmatic hernia is well recognized.[13] In this series there was evidence of congenital heart disease in 16.4% of cases; this was associated with a poor outcome. There were only two fetuses (3.6%) with a karyotype anomaly, which is a much lower rate than that reported in other series.[6, 14] This is presumably because most of our patients were referred after the result of chromosomal analysis was known and the patient was planning to continue the pregnancy.

The main cause for the high mortality associated with this condition is pulmonary hypoplasia.[15] Prenatal therapy has been aimed at trying to prevent this from occurring.[16] It is difficult to assess the critical mass of lung tissue required for survival. Infants survive resection of a malformed or infarcted lung, suggesting that adequate ventilation is possible with 50% of normal lung mass. Infants dying with a large diaphragmatic hernia usually have a lung weight of 15% to 40% of expected weight and it has been sugested that the critical mass is between the two.[17] To date, there have been no accurate methods defined for estimating fetal lung mass prenatally. It has been suggested that the area of the hernia can be measured on ultrasonography and related either to the area of the chest or to the size of the heart.[7, 10] The area of the hernia in the chest is not always clearly defined on ultrasonographic scanning. so that an overestimation or underestimation may occur. Further studies are required to establish whether lung volume can be accurately predicted prenatally. For the present time, it would be easier to assess the secondary effects on the cardiac structures when attempts are made to predict the outcome.

In conclusion, although the overall mortality associated with fetal congenital diaphragmatic hernia is high, the odds for survival are significantly improved if other abnormalities are excluded and there are few or no signs of left heart underdevelopment. Thus examination of the fetal heart is important in the evaluation of each case, both in terms of detecting structural cardiac abnormalities and for assessing the development of the cardiac chambers. Careful counseling of the parents is required, particularly in cases seen in early pregnancy, as the potential for change with advancing gestational age must be taken into account. Termination of pregnancy is an option before 24 weeks of gestation. In cases that continue to term, sequential observations may be helpful in selecting cases likely to be associated with a good outcome. In these cases careful attention to postnatal perioperative management is required, and the influence of this on the outcome needs further assessment before prenatal therapy is contemplated.

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Abruptio placentae associated with perforated appendicitis and generalized peritonitis

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A primigravid woman at 35 weeks' gestation was admitted with abdominal pain, fever, and vomiting. Forceful contractions and signs of fetal distress suggested abruptio placentae. During cesarean section, seropurulent exudate and a perforated appendix were found; an appendectomy was performed. A mechanism linking appendicitis with abruptio placentae is suggested. (AM J OBSTET GYNECOL 1992;166: 14-5.)

Key words: Abruptio placentae, acute appendicitis, peritonitis

A case of abruptio placentae occurring in association with a perforated appendix is described.

Case report

A 26-year-old primigravid women was hospitalized at 35 weeks of gestation because of the sudden onset of abdominal pain associated with copious vomiting. Before admission the clinical and ultrasonographic follow-up indicated an uneventful pregnancy with an appropriate-for-gestational-age, normally developing fetus. There was no hypertension, diabetes mellitus, or history of external trauma. The pateint did not smoke or consume alcohol, nor was she a drug abuser.

On admission, the patient was apprehensive and tachypneic. Temperature was 37.8° C, pulse 92 beats/min and blood pressure 100/60 mm Hg. On examination, the uterine size was appropriate for gestational age with evident tenderness above it and alongside, predominantly on the right. The fetus was in a vertex presentation, and the cervix was 2 cm long, firm, and dilated to 1.5 cm. On ultrasonography, the biophysical profile was normal, and the placenta was at the fundus with no apparent signs of abruptio placentae. On cardiotocography, fetal heart rate was normal and reactive. Hemoglobin was 13 gm/dl, white blood cell count 11,700 mm^3, and blood glucose, urea, electrolytes, and clotting factors were within the normal range. Urinalysis results were normal. Within 4 hours the patient complained of contractions with increasing frequency and intensity. Shortly thereafter the uterine tone ceased to subside between contractions, but the fetal heart rate remained normal and reactive. The cervix was then effaced and dilated to 2 cm. Abruptio placentae was suspected and amniotomy was performed. The fluid was clear, and on direct internal tocography the basal uterine pressure was 30 to 40 mm Hg. Epidural analgesia was established, and ampicillin and gentamicin therapy was initiated. In the following 2 hours the cervix dilated to 3 cm; at that time blood clots first appeared through the cervix. The fetal heart rate pattern then showed late decelerations, and a cesarean section was performed. In the peritoneal cavity 300 ml of odorless, seropurulent fluid was present. A 2425 gm male infant was delivered, with Apgar scores of 7 and 9 at 1 and 5 minutes, respectively. The placenta followed immediately, with numerous blood clots covering about 30% of its surface. Exploration of the abdominal cavity revealed a perforated appendix with a fecalith at the perforation site and leakage of pus. Appendectomy was performed, and the peritoneal cavity was thoroughly rinsed. The postoperative period was uneventful.

Pathologic examination of the appendix was consistent with acute appendicitis. *Escherichia coli* was cultured in specimens from the appendix, peritoneal fluid, and placenta. Culture specimens from the neonate's nose, pharynx, and ear canals were sterile.

Comment

The combination of acute appendicitis and abruptio placentae poses a difficult diagnostic problem. Nevertheless, the presence of both conditions is unlikely to be coincidental. A possible explanation linking the events beginning with acute appendicitis and culminating in a premature separation of the placenta is presented.

With maternal intraperitoneal infection, microorganisms may gain access to the intervillous space by hematogenous dissemination during maternal bacteremia. From there, the infection could spread to the villi and fetal circulation. Bacterial colonization of the decidua and fetal membranes without clinical evidence of infection was reported. Darby et al.[1] reported that histologic chorioamnionitis and funisitis were present

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significantly more often in patients with acute, severe preterm abruptio placentae than in normal deliveries. They speculated that direct bacterial colonization of the decidua with resultant tissue inflammation may initiate processes, such as disruption of decidual lysosomes, that ultimately result in abruptio placentae.

Whereas direct bacterial colonization of the decidua is a possibility when generalized peritonitis is present, this need not necessarily occur, as dissemination of bacterial endotoxin is also capable of initiating similar processes. The initiation of normal parturition is dependent on activation of the decidua and the production of prostaglandins by accelerated hydrolysis of glycerophospholipids.[2] It has been shown that the decidua responds to bacterial infection by increased biosynthesis of prostaglandin, stimulated either by bacterial endotoxin or by host signals (tumor necrosis factor, interleukin-1, platelet-activating factor) that were secreted in response to the presence of microbes.[2]

In the case under discussion, generalized peritonitis caused by a ruptured appendix may have been associated with dissemination of bacteria and bacterial endotoxin that stimulated prostaglandin biosynthesis in excess. Both increased prostaglandin biosynthesis and direct colonization of the placental villi with tissue disruption could have resulted in abruptio placentae.

In summary, we suggest that in pregnancy an acute bacterial infection such as generalized peritonitis may cause abruptio placentae.

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The effect of continuous subdermal levonorgestrel (Norplant) on carbohydrate metabolism

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Blood glucose and plasma insulin levels during an oral glucose tolerance test were measured in 20 women using continuous subdermal levonorgestrel (Norplant) for contraception over a 12-month period. Changes in carbohydrate metabolism occurred as early as 1 month but were most marked after 6 months. After 1 month the area under the glucose and insulin curves increased by 12.3% and 37.7%, respectively. This increase was more marked by 6 months, but by 12 months it was significantly less marked. Although changes in the blood glucose and plasma insulin levels at different times during the oral glucose tolerance test occurred, they were all within normal limits for normal women. The peak blood glucose and insulin levels after insertion of the implant occurred 60 minutes after the glucose load, as opposed to 30 minutes before insertion. Apart from this, there was a significant delay in the return of these levels to fasting values. We conclude that this product, like any other progesterone-containing contraceptive, alters carbohydrate metabolism, but these alterations are not clinically significant in normal women. It is, however, possible that in potential cases of diabetes it may predispose to frank diabetes. (Am J Obstet Gynecol 1992;166:15-9.)

Key words: Norplant, levonorgestrel, progestogen, carbohydrate metabolism

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6/1/31816

Hormonal contraceptives remain one of the most effective reversible methods of family planning worldwide. Although first marketed in 1957, it was not until the pioneering work of Waine et al.[1] that attention was drawn to the adverse metabolic effects of these hormones. It is now generally believed that the altered

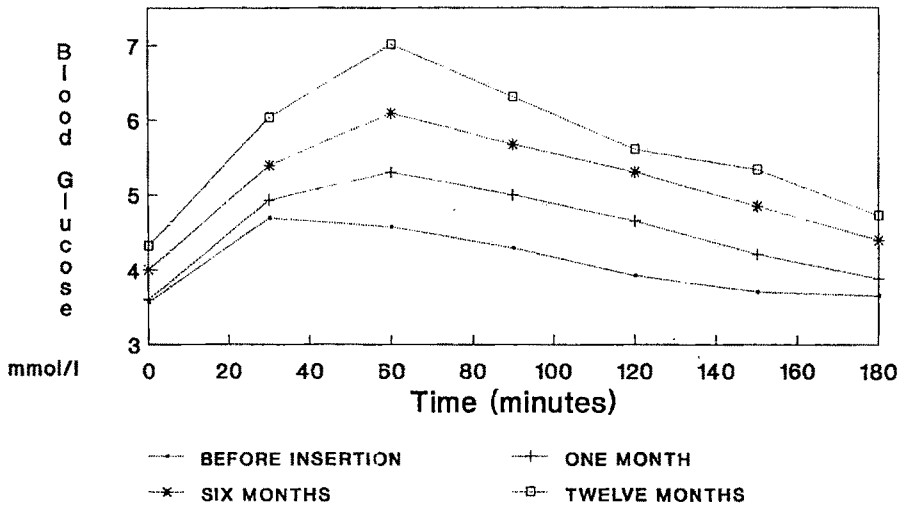


Fig. 1. Mean blood glucose values during GTT in 20 Norplant users over a 12-month period.

carbohydrate metabolism in women using combination oral contraceptives is mainly due to the progestogen component.[2-7] Of the various classes of progestogens used for contraception, the 19-norprogestogens alter carbohydrate metabolism most.[3,5] Levonorgestrel belongs to this potent group and has no doubt been extensively investigated.

As research continues on adverse metabolic effects of various hormonal contraceptives, so too are attempts to develop long-acting preparations and delivery systems that will improve effectiveness, safety, and acceptability. The continuous subdermal implant Norplant is one of these new long-acting contraceptives containing the 19-norprogestogen levonorgestrel. Regulatory approval has been given for its marketing in many developing and developed countries,[8,9] and very recently by the Food and Drug Administration in the United States. Unlike oral contraceptive pills and injectable contraceptives, the release of levonorgestrel peaks to 80 μg/24 hours during the first weeek after insertion and declines steadily to stabilize at 30 μg/24 hours after 12 to 18 months.[10,11] While lipid and coagulation changes have been extensively studied in Norplant users,[12-15] such changes in carbohydrate metabolism have not been properly studied. The aim of this study was therefore to monitor the alterations in carbohydrate metabolism that occur in Norplant users over a 12-month period.

Material and methods

Twenty women voluntarily seeking contraceptive advice at the University College Hospital, Ibadan, Nigeria, family planning clinic, who opted for Norplant after counseling and satisfied the inclusion criteria, were recruited into the study. These criteria were age 20 to 40 years, regular menstrual cycles in the last 12 moths, absence of known or suspected medical disorders, weight between 40 and 80 kg, nonsmoker, and not receiving steroids, antibiotics, barbiturates, and antituberculous drugs. All those who had either been pregnant or used other hormonal contraceptives in the preceding 12 months were excluded. Signed consent was obtained from each patient, and approval for the study was obtained from the Joint Ethical Committee of the University of Ibadan and University College Hospital, Ibadan, Nigeria.

Glucose tolerance test. An average Nigerian diet contains at least 60% carbohydrate; hence no special dietary preparation was required. After a 12-hour overnight fast, an oral glucose tolerance test (GTT) was performed at 8 AM, before Norplant insertion. After the patient rested in a chair for 30 minutes, an intravenous cannula (20 Fr) was inserted into the antecubital vein, and 5 ml of blood was withdrawn. Three milliliters was placed in a heparinized tube and later centrifuged. The serum was then separated and labeled. The remaining 2 ml was placed in an oxalate fluoride bottle and also labeled. Each patient was then given a solution of 250 ml of water containing 75 gm glucose to drink. Repeat venous samples were collected at 30-minute intervals for 3 hours and separated as detailed. The seven samples for glucose estimation were immediately transported to the laboratory, where blood glucose was estimated with the glucose oxidase method on a Beckman glucose autoanalyzer (Beckman Instruments, Inc, Fullerton, Calif.). Serum for insulin was stored at −20° C and later measured with Amersham radioimmunoassay kits (Abbott Laboratories, North Chicago). The measurements for each patient were performed as single assays.

As soon as the oral GTT was completed, six capsules of Norplant were inserted on the medial aspect of the

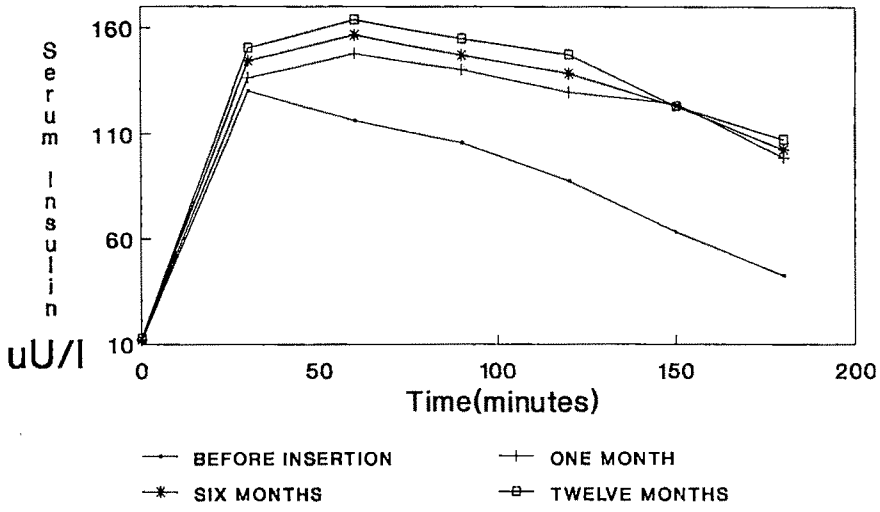


Fig. 2. Mean serum insulin values during GTT in 20 Norplant users over a 12-month period.

Table I. Mean blood glucose values for 20 Norplant users over a 12-month period

Duration of Norplant use (mo)	*Glucose values (mmol/L, mean ± SD) during oral GTT*						
	Fasting	*30 min*	*60 min*	*90 min*	*120 min*	*150 min*	*180 min*
0 (Before insertion)	3.57 ± 0.46	4.69 ± 0.86	4.57 ± 0.67	4.29 ± 0.62	3.92 ± 0.77	3.70 ± 0.65	3.65 ± 0.75
1	3.60 ± 0.46 $t = 0.2$ $p > 0.5$	4.93 ± 0.93 $t = 0.857$ $p > 0.5$	5.30 ± 1.07 $t = 2.607$ $p < 0.01$	5.00 ± 0.70 $t = 3.550$ $p < 0.001$	4.65 ± 0.85 $t = 2.808$ $p < 0.02$	4.20 ± 0.68 $t = 2.500$ $p < 0.05$	3.88 ± 0.74 $t = 0.958$ $p < 0.5$
6	4.00 ± 0.62 $t = 2.529$ $p < 0.02$	5.39 ± 1.10 $t = 2.244$ $p < 0.05$	6.09 ± 1.19 $t = 5.017$ $p < 0.001$	5.67 ± 0.92 $t = 5.565$ $p < 0.001$	5.30 ± 0.83 $t = 5.541$ $p < 0.001$	4.84 ± 1.17 $t = 3.810$ $p < 0.001$	4.39 ± 0.63 $t = 3.379$ $p < 0.01$
12	4.32 ± 0.69 $t = 4.412$ $p < 0.001$	6.03 ± 1.23 $t = 4.295$ $p < 0.001$	7.01 ± 1.02 $t = 0.037$ $p < 0.0001$	6.31 ± 0.77 $t = 9.140$ $p < 0.0001$	5.60 ± 0.78 $t = 6.855$ $p < 0.0001$	5.33 ± 0.65 $t = 7.932$ $p < 0.0001$	4.72 ± 0.67 $t = 4.758$ $p < 0.001$

The p value is statistical significance for glucose value compared with the corresponding value before insertion.

upper arm with the method recommended by Alvarez.[16] The oral GTT was then repeated at 1, 6, and 12 months after insertion. Blood glucose and serum insulin values and the areas under the curves were compared with the paired t test.

Results

The mean age and parity of the 20 patients were 32.9 ± 3.9 (range 25 to 39 years) and 5.8 ± 1.5 (range 4 to 8). The mean weight at admission was 56.7 ± 9.4 kg but changed to 56.6 ± 9.3, 56.8 ± 9.4, and 57.9 ± 9.6 kg at 1, 6, and 12 months after Norplant insertion. These weight changes were small and insignificant.

Blood glucose. The mean blood glucose values during oral GTT over the 12-month period are shown in Table I and depicted graphically in Fig. 1. There was a consistent rise in the various blood glucose values after 1, 6, and 12 months on Norplant. The rise in blood glucose level at various times during the oral GTT was more marked after 6 and 12 months on Norplant than after 1 month. The p values for these changes are shown in Table 1.

The peak blood glucose level during oral GTT occurred at 30 minutes before Norplant insertion, but it occurred at 60 minutes after Norplant insertion. There was also a slower return of the blood glucose value to fasting levels after Norplant insertion.

The area under the oral GTT curve was 247.7 ± 19.4 before insertion (AG_0) but rose to 278.20 ± 23.7 (AG_1), 314.85 ± 31.8 (AG_6), and 347.85 ± 44.3 (AG_{12}) after 1, 6, and 12 months on Norplant, respectively. The changes in area from insertion to 1, 6, and 12 months afterward were 30.50, 67.15, and 100.25, respectively. These represented 12.3%, 25.0%, and 40.4% increases, respectively. The $\Delta AG_{0\text{-}6}$ and $\Delta AG_{0\text{-}12}$ values were statistically greater when compared with $\Delta AG_{0\text{-}1}$ ($p < 0.05$ and $p < 0.01$, respectively).

Serum insulin. Table II shows the changes in mean

Table II. Mean serum insulin values for 20 Norplant users over a 12-month period

Duration of Norplant use (mo)	*Serum insulin values (μU/ml, mean ± SD) during oral GTT*						
	Fasting	*30 min*	*60 min*	*90 min*	*120 min*	*150 min*	*180 min*
0 (Before insertion)	11.6 ± 3.5	130.4 ± 13.2	116.3 ± 9.5	105.7 ± 8.7	87.6 ± 8.4	63.4 ± 7.3	42.7 ± 6.3
1	12.0 ± 4.0	136.5 ± 8.7	148.0 ± 10.3	140.4 ± 9.7	129.7 ± 9.5	120.5 ± 6.3	93.8 ± 6.1
	$t = 0.3367$	$t = 1.7256$	$t = 10.118$	$t = 11.910$	$t = 14.847$	$t = 26.484$	$t = 28.609$
	$p > 0.5$	$p < 0.05$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
6	12.2 ± 4.1	144.7 ± 6.2	156.6 ± 11.3	147 ± 10.8	138 ± 8.3	124 ± 5.1	102 ± 5.6
	$t = 0.4979$	$t = 4.3852$	$t = 12.212$	$t = 13.323$	$t = 19.087$	$t = 30.434$	$t = 31.462$
	$p > 0.5$	$p < 0.001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
12	12.7 ± 4.5	150.6 ± 5.7	163.7 ± 9.4	155 ± 10.5	147 ± 9.2	123 ± 7.9	107 ± 8.5
	$t = 0.8629$	$t = 6.283$	$t = 15.363$	$t = 16.169$	$t = 21.234$	$t = 24.782$	$t = 27.180$
	$p > 0.5$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

The p value is statistical significance for insulin value compared with corresponding value before insertion.

serum insulin levels at various times over 12 months of Norplant use. The changes are also shown graphically in Fig. 2. Again there is a consistent rise in various values at different times of the oral GTT. Unlike blood glucose values, there was a very small rise in fasting insulin levels. The peak insulin value was measured 30 minutes before Norplant insertion and 60 minutes after insertion. The return of serum insulin to fasting levels was slower after Norplant insertion.

The area under the insulin curves rose from 530.6 ± 120.4 (IA_0) at insertion to 730.5 ± 170.6 (IA_1) at 1 month, 768.7 ± 169.5 (IA_6) at 6 months, and 799.9 ± 179.2 (IA_{12}) at 12 months after insertion. The change in area from insertion to 6 months afterward (ΔI_{0-6}) was 238.1 and from insertion to 12 months after was (ΔI_{0-12}) 269.3, representing a 44.9% and a 50.8% rise from the area at insertion (IA_0), respectively. The increase in area was 199.9 from insertion to 1 month afterward (ΔI_{1-0}) and 31.2 from 6 to 12 months after insertion (ΔI_{6-12}), representing a 37.7% and a 4.1% rise, respectively.

Comment

Since the pioneering work of Waine et al.[1], various studies have been conducted to determine the secondary metabolic effects of steroidal contraceptives.[1-6] Most of these have shown that the gonane progestogens are the most potent in altering carbohydrate metabolism.[3,5] Almost all the previous studies have been with oral contraceptives. Administration of these hormones is associated with daily fluctuations in serum progestogen levels. In addition, ever-changing conditions of the gastrointestinal and hepatic systems may affect absorption and metabolism. The daily dose of progestogens in most products varies from 150 μg to 2 mg, but levonorgestrel release from Norplant rises to 80 μg within the first week and drops steadily to 30 μg after 12 to 18 months. We aimed in this study to determine the extent to which continuous levonorgestrel, when given alone, will alter carbohydrate metabolism.

Our results show that alteration in carbohydrate metabolism with continuous low-dose levonorgestrel occurs as early as 1 month after insertion. Although changes in glucose and insulin curves occurred throughout the 12-month period, it was more marked over the first 6 months. This would suggest that the alteration in carbohydrate metabolism is partly dependent on the dose of levonorgestrel released. We feel that these alterations may in fact stabilize by 18 months when levonorgestrel release stabilizes at 30 μg/24 hours.

Although there was an alteration in carbohydrate metabolism, none of the glucose tolerance curves were either diabetic or suggestive of impaired glucose tolerance. This is in contrast with the findings of Spellacy et al.[4] on 19-norgestrel where 18% of the glucose tolerance test results were abnormal after 18 months. This difference may be due to the dose of levonorgestrel administered.

Whereas the rise in fasting blood glucose level after 6 and 12 months on Norplant in our study was statistically significant, others have reported either no rise[17-19] or a statistically insignificant rise.[20,21] Dietary and cultural differences might be responsible. The glucose and insulin curves showed a change in pattern after Norplant insertion. Similar changes have also been noted by others but with other forms of hormonal contraceptives.[4,6]

Although extensive research has been conducted on the alterations in carbohydrate metabolism caused by hormones, the exact mechanism of these alterations is yet to be universally agreed on. We speculate that the delayed peaking of the glucose and insulin curves in our cases might have been due to delayed absorption of glucose from the gastrointestinal tract. It is also possible that decreased gastrointestinal motility induced by the progestogens may be a contributing factor. Other mechanisms that have been suggested for these changes include alteration in liver morphologic characteristics and function,[22] increase in peripheral insulin resis-

tance,[23] and increase in insulin antagonistic hormones such as growth hormone, cortisol, and glucagon.[24] Diaz et al.,[25] however, in studying cortisol, thyroid, and prolactin changes in Norplant users, found no such increases. Whatever the mechanism, 17-estradiol, when administered with progestogens, is said to enhance these changes.[7] Therefore the absence of estrogen in Norplant may be partly responsible for the milder alterations.

Although we have demonstrated that Norplant induces changes that are not clinically significant in normal patients, such changes in diabetes or potential diabetes may have significant clinical implications. There is likely to be a need for increased insulin requirements, and those with impaired carbohydrate metabolism may become overtly diabetic. We therefore suggest that until more studies are carried out on diabetic women and others at risk these conditions should be a relative contraindication. Where there is no alternate form of contraception, Norplant may be used but the diabetic control will need to be monitored more carefully.

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Familial occurrence of thrombotic thrombocytopenic purpura in two sisters during pregnancy

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The etiology of thrombotic thrombocytopenic purpura is not fully understood. A genetic predisposition and other inciting factors have been suggested by several studies to bring about the full-blown syndrome. We report two cases of thrombotic thrombocytopenic purpura in two sisters with sudden onset during late pregnancy. (AM J OBSTET GYNECOL 1992;166:20-1.)

Key words: Thrombotic thrombocytopenic purpura, pregnancy, heredity

Thrombotic thrombocytopenic purpura is a well-recognized clinical syndrome associated with diverse conditions. Various studies suggested that genetic factors and immunologic and infectious causes can induce this disorder to appear. A combination of genetic factors with a precipitating event, pregnancy, was previously suggested by Wallace et al.[1] Fuchs et al.[2] described two sisters in whom acute and fatal thrombotic thrombocytopenic purpura occurred during their first pregnancies. We report here the second case of a classic syndrome of thrombotic thrombocytopenic purpura that occurred during late pregnancy in two primiparous sisters. In each sister the disease began suddenly, and one of the sisters is alive.

Case reports

Case 1. In 1980, a 19-year-old patient, gravida 1 para 0, was hospitalized at her thirty-first week of gestation complaining of generalized weakness and vomiting. Her medical history was uneventful.

Physical findings revealed a well-developed and well-nourished woman, appearing pale and diaphoretic with diffused purpura on both legs. Blood pressure was 120/80 mm Hg, pulse was 88 beats/min, and she had no fever. The uterus was appropriate for gestational age, with the fetus in vertex presentation. Immediately after admission she convulsed. The initial laboratory evaluation revealed microangiopathic hemolytic anemia, thrombocytopenia of 10,000/mm^3, hemoglobin of 5.8 gm/dl, and urinalysis showing macrohematuria. Six hours after the grand mal seizure, the fetus died and labor was induced. Thereafter, sudden deterioration in the mother's condition occurred and she subsequently died. Autopsy examination showed thrombi in the small vessels of the heart, brain, lungs, and glomerular capillaries consistent with the diagnosis of thrombotic thrombocytopenic purpura.

Case 2. In 1990 the 26-year-old sister of patient No. 1 was seen during the twenty-seventh week of her first pregnancy in a stuporous state with purpura on both legs. Her prenatal course was complicated by repeated urinary tract infections with *Escherichia coli*. On admission the examination revealed a restless, stuporous, toxic-appearing woman with a temperature of 39.6° C. Blood pressure was 110/80 mm Hg and pulse 120 beats/min. The uterus was appropriate for gestational age, with the fetus in vertex presentation. The uterine cervix admitted a fingertip but was not effaced. Initial laboratory workup revealed a white blood cell count of 4300 cells/mm^3, hemoglobin of 7.6 gm/dl, and platelet count of 66,000/mm^3. Blood coagulation profile was within normal limits. Blood and urine specimens both revealed *E. coli*. Four hours after her admission, two grand mal seizures occurred.

A cesarean section was performed because of severe fetal bradycardia, and a 1100 gm male infant with an Apgar score of 2 at 1 minute was delivered. The infant died within a few minutes.

Amniotic fluid cultures obtained during the cesarean section demonstrated the same serotype of *E. coli* that was isolated from maternal blood and urine. Postoperatively, the patient was bleeding through the nasogastric tube and at the central venous pressure catheter site. Further laboratory evaluation showed a decrease in platelet count to 15,000/mm^3, a few fragmented red blood cells on the peripheral smear, a negative test result for antinuclear antibody, and normal prothrombin time, partial thromboplastin time, and fibrinogen levels. Serum creatinine concentration rose to 8 mg/dl. The patient was transferred to the general intensive care unit, where she was treated for 41 days for multiple life-threatening conditions. At admission she was stuporous, hypothermic (33° C) and rapidly developed septic shock. A bleeding diathesis consistent with disseminated intravascular coagulation improved after

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massive transfusion of blood, plasma, and several clotting factors. Two days after admission to the intensive care unit the patient became fully alert and conscious.

On the fifth day, the patient again became lethargic and stuporous and signs of meningeal irritation and right hemiparesis developed. Brain computed tomographic scan showed a large left frontoparietal infarct without signs of mass effect or edema. On the seventh day, the patient's condition deteriorated into deep coma. There was renal failure with creatinine levels of 10 mg/dl and urea levels of 550 mg/dl.

Eleven days after admission, the patient had a repeated event of septic shock. Pulmonary artery catheterization showed a low-resistance, high-cardiac-output failure. Cultures from the sputum and urine grew *Acinetobacter* and drainage of a large pleural effusion grew *Pseudomonas aeruginosa.*

After aggressive antibiotic and steroid treatment the patient's status gradually improved. She regained consciousness with neurologic and renal function slowly returning to normal.

On the forty-first day of hospitalization, the patient recovered completely and was discharged from the hospital to be followed up monthly at the outpatient clinic. Sixteen months later she is healthy and without any pathologic sequelae.

Comment

Thrombotic thrombocytopenic purpura is an acute disease of unknown cause. In some patients pregnancy appears to exacerbate or trigger the onset.

Hereditary factors may play a role in etiology and pathogenesis. Thrombotic thrombocytopenic purpura has been reported before in siblings.[1] Moreover, Fuchs et al.[2] have reported the classic syndrome of fatal thrombotic thrombocytopenic purpura in two sisters during pregnancy. The authors concluded that this disorder may have a genetic predisposition that is triggered during late pregnancy by various inciting factors, such as sepsis. In both sisters thrombotic thrombocytopenic purpura occurred at almost the same stage of their first pregnancies, the clinical course was similar, and the triggering factor was associated with infections. The recovery of the second sister can be attributed to the early diagnosis and aggressive intensive care.

Physicians and patients should be alerted to the possibility of familial occurrence of thrombotic thrombocytopenic purpura during pregnancy. Aggressive treatment of an infectious disease should be initiated as soon as possible.

Addendum

A third sister became pregnant, and her pregnancy was complicated with thrombocytopenia. Because of the family history and the physician's alertness to this rare disease, a prompt diagnosis of thrombotic thrombocytopenic purpura was made.

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Numeric analysis of heart rate variation in intrauterine growth–retarded fetuses: A longitudinal study

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OBJECTIVE: We attempted to determine changes occurring with time in fetal heart rate and its variation in fetuses with intrauterine growth retardation in whom late antepartum fetal heart rate decelerations eventually develop.

STUDY DESIGN: Thirteen fetuses with intrauterine growth retardation were studied over a median period of 25 days. One-hour fetal heart rate records were made two to five times per week and were analyzed numerically. Fetal movements were recorded by the women.

RESULTS: On average long-term fetal heart rate variation decreased gradually with time and fell below the norm (30 milliseconds) at about the same time decelerations appeared. Mean heart rate showed a slight but statistically significant increase after the occurrence of decelerations. There were large interfetal differences in all parameters studied.

CONCLUSION: In fetuses with intrauterine growth retardation a decrease in long-term fetal heart rate variation is a rather late sign of impairment that coincides with the occurrence of late decelerations. In the surveillance of the fetus with intrauterine growth retardation it might be most appropriate to use each fetus as its own control. (AM J OBSTET GYNECOL 1992;166:22-7)

Key words: Intrauterine growth retardation, fetal heart rate monitoring, fetal hypoxemia, computer analysis

In cross-sectional studies of fetuses with intrauterine growth retardation (IUGR), long-term heart rate variation is usually within the normal range in the absence of antepartum late fetal heart rate (FHR) decelerations, but it is reduced in the presence of late decelerations.[1, 2] Reduced FHR variation and the occurrence of decelerations coincide with hypoxemia, as determined in cord blood obtained by cordocentesis or at elective cesarean section,[2-4] and are assumed to precede the "terminal" state, in which fetal acidemia is present.[5, 6] These data suggest that a reduction in heart rate variation is a rather late sign of impairment of the fetal condition.

The cross-sectional design of these studies has not allowed determination of the actual sequence of changes in FHR patterns with progressive deterioration. In this study therefore heart rate and its variation were measured longitudinally in fetuses with IUGR to determine changes occurring with time and progressive deterioration.

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Patients and methods

The study group consisted of 13 women admitted to the obstetric ward. They were selected retrospectively on these criteria: (1) Computerized analyses of 1-hour FHR records were available before and after the occurrence of antepartum late decelerations; (2) cesarean section had been performed because of recurrent late decelerations; (3) the infants had a birth weight below the 10th percentile as corrected for sex and parity[7] and had no congenital malformations. All of the women had been admitted because of the clinical diagnosis of IUGR. Nine had pregnancy-induced hypertension; proteinuria was detected in four women. Four patients were treated with antihypertensive drugs, and two received 80 mg of acetylsalicylic acid daily. No other medication was prescribed.

The clinical data of the infants are shown in Table I. Most were delivered before 35 weeks of gestation. All but one (case number 12) had abnormal umbilical artery blood velocity waveforms, and in seven there was absence of end-diastolic frequencies above filter level (150 Hz). In one there was reversed flow during diastole. In the seven fetuses in whom blood velocity waveforms were measured in the internal carotid artery, the pulsatility index (Peak systolic velocity − Peak diastolic velocity/Mean velocity) was decreased, which probably indicated reduced cerebral vascular resistance. The blood velocity waveforms in the umbilical artery and internal carotid were related to observations made on normal fetuses.

Table I. Clinical data of 13 fetuses with IUGR*

Case No.	Gestational age: First deceleration	Gestational age: At cesarean section	Head/abdomen ratio	Pulsatility index: Umbilical artery	Pulsatility index: Internal carotid artery	Mean minute range (msec)	Birth weight: gm	Birth weight: %	Umbilical artery: pH	Umbilical artery: Po_2 (kPa)
1	29 wk 0 days	29 wk 3 days	1.24	2.93†	—	34.3	975	<5	7.11	—
2	30 wk 1 day	30 wk 6 days	1.18‡	2.05†	0.92	18.7	900	<2.3	7.17	—
3	30 wk 5 days	31 wk 0 days	1.26	2.93§	0.96	12.9	900	<2.3	7.25	2.22
4	30 wk 4 days	31 wk 3 days	1.26	1.93†	—	19.1	890	<2.3	7.21	0.97
5	32 wk 6 days	32 wk 6 days	1.12‡	2.71†	—	33.8	1245	<5	7.22	—
6	31 wk 6 days	33 wk 0 days	1.11‡	2.35†	—	22.0	1035	<2.3	7.22	1.35
7	31 wk 4 days	33 wk 6 days	1.18	1.71	1.08	33.2	1130	<10	7.21	3.44
8	33 wk 6 days	33 wk 6 days	1.16	1.59	0.93	40.1	1310	<2.3	7.28	2.70
9	31 wk 2 days	34 wk 0 days	1.09‡	2.71	0.91	23.2	1160	<2.3	7.16	1.05
10	33 wk 1 day	34 wk 4 days	1.17	2.62†	0.75	22.4	1150	<2.3	7.30	0.62
11	34 wk 2 days	35 wk 0 days	1.12	2.10	—	24.9	1490	<5	7.30	2.23
12	34 wk 5 days	36 wk 5 days	1.26	1.10	0.97	25.3	1750	<2.3	7.26	1.70
13	35 wk 5 days	37 wk 6 days	1.24	1.44	—	59.2	1735	<2.3	7.22	—

*Data on head/abdomen ratio, pulsatility index (absolute values), and mean minute range refer to the last measurement before cesarean section.

†Absence of end-diastolic velocities.

‡Symmetric IUGR.

§Reversed flow.

Before birth, nine fetuses exhibited asymmetric growth retardation; four were symmetrically growth retarded, according to the head/abdomen circumference ratios.[8] At birth, nine infants weighed <2.3rd percentile. Five of the nine fetuses with measurements had hypoxemia (umbilical arterial $Po_2 < 2.00$ kPa) and in 10 of the 13 fetuses the pH was within the normal range (umbilical artery pH > 7.20).[2] There was one neonatal death. Other complications included intraventricular hemorrhage ($n = 1$), necrotizing enterocolitis ($n = 1$), and hyaline membrane disease ($n = 4$).

In the 13 fetuses 200 1-hour FHR records were made with a Hewlett Packard model 8040A cardiotocograph over a median period of 25 days (range 4 to 44 days) two to five times per week between 10 AM and 6 PM. The records were analyzed numerically with an on-line microcomputer system[9] adapted for use with a personal computer (Apricot Xen). This computer program for FHR analysis is identical to that of the commercially available Sonicaid System 8000. With this system a baseline is fitted to the FHR trace. Accelerations (≥ 10 beats/min lasting ≥ 15 seconds) and decelerations (≥ 10 beats/min lasting ≥ 60 seconds or ≥ 20 beats/min lasting ≥ 30 seconds) are then identified. FHR variation is measured as the range of mean pulse intervals about the baseline calculated minute by minute, with data from decelerations excluded; it is averaged to give a measure of long-term variation ("mean minute range" in milliseconds). Episodes of high and low heart rate variation are identified when 5 of 6 successive minutes have a range of pulse intervals > 32 or < 30 milliseconds, respectively. Basal FHR is calculated as the mean FHR in episodes of low variation or, if no such episodes are present, as the mean pulse interval expressed as beats per minute.

The fetal movements felt by the women were recorded with an event marker. No more than one movement every 1/16 minute was identified. An example of a 1-hour recording is shown in Fig. 1. The number of records differed among the fetuses; therefore data were standardized per fetus per week (Figs. 2 and 3).

It has been shown previously that FHR variation is > 30 milliseconds in 95% to 97% of normal third-

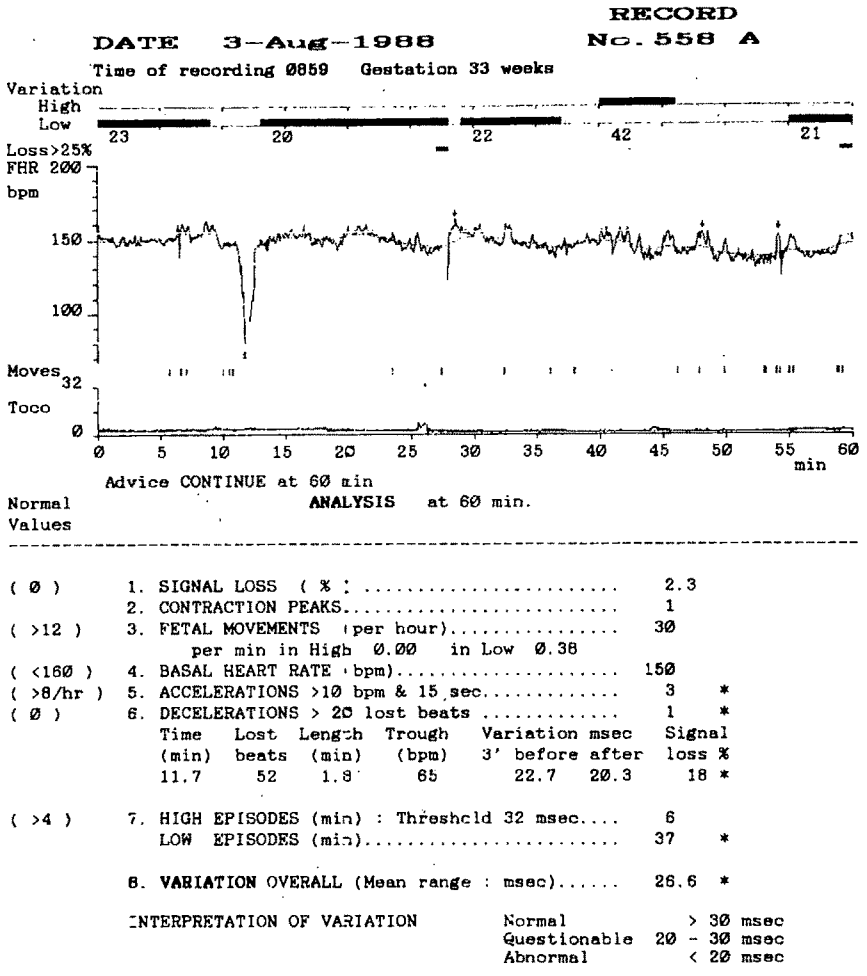


Fig. 1. Example of computer printout of 1-hour record at 33 weeks. Heart rate variation is below normal range (26.6 milliseconds); there is one large deceleration.

trimester fetuses.[9] This value was taken as the lower limit of normality. Because neither this lower limit nor that of the incidence of fetal body movements[2] changes during the third trimester of pregnancy, the data were grouped according to the first occurrence of late decelerations without regard to the differences in gestational age. The occurrence of antepartum late decelerations was assessed visually from the original FHR traces[5, 6] by clinicians not aware of the data from the numeric analysis. The women were delivered of their infants between <1 day and 19 days after the first deceleration occurred (median interval 6 days). On average, visually assessed decelerations were present in 38% of the records made after the occurrence of the first deceleration.

Results

The changes with time in basal heart rate, FHR variation, and the percentage of time that episodes of high FHR variation were present are shown in Fig. 2. There was a slight but significant increase in basal FHR when decelerations occurred (Wilcoxon test, $p < 0.05$). On average, FHR variation decreased gradually with time. The median value fell below the normal range (30 milliseconds) at about the time at which decelerations appeared. A similar gradual decline occurred in the duration of episodes of high FHR variation (Fig. 2) and in the incidence of fetal movements as felt by the women (Fig. 3). The number of accelerations per hour was low initially and remained constant (Fig. 3).

There were no differences among fetuses with symmetric and asymmetric IUGR except for the incidence of movements felt subjectively, which was somewhat higher in the fetuses with symmetric IUGR.

Large interfetal differences were present (Figs. 2 and 3). In Figs. 4 and 5 basal heart rate, FHR variation, and episodes of high variation are shown for two fetuses delivered at 37 weeks and for two delivered at 31 weeks. In spite of similar birth weights there were large differences in FHR variation, which was already low in one fetus weeks before the onset of decelerations; in

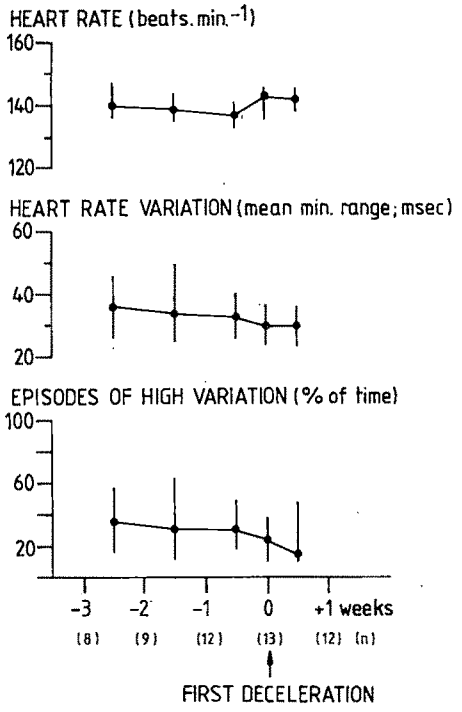


Fig. 2. Changes with time in basal heart rate, heart rate variation, and percentage of time in which episodes of high heart rate variation are present in 13 growth-retarded fetuses. Data are grouped according to first occurrence of late decelerations and are presented as medians and interquartile ranges. Numbers in parentheses are number of fetuses included.

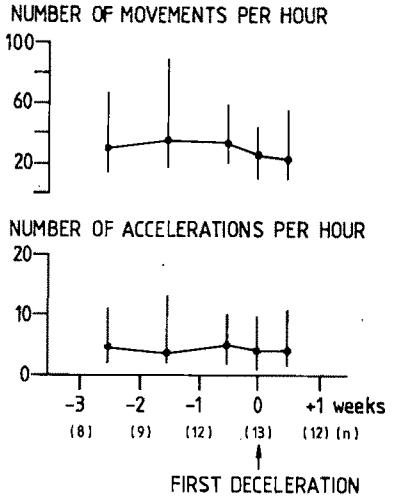


Fig. 3. Changes with time in number of movements felt by women and in number of accelerations (≥ 10 beats/min lasting ≥ 15 milliseconds) in 13 growth-retarded fetuses. Data are grouped according to first occurrence of late decelerations and are presented as medians and interquartile ranges.

the other it was still high after the onset of decelerations (Fig. 4) or decreased when the first deceleration occurred (Fig. 5). All four fetuses in Figs. 4 and 5 had asymmetric growth retardation; no antihypertensive drugs were administered.

There was a gradual increase in the pulsatility index of the umbilical artery (Fig. 6). The pulsatility index was increased (> 2 SD above the mean) in 7 of 10 cases studied 3 weeks before the decelerations occurred, in 7 of 12 cases studied at −2 weeks, in 10 of 13 cases at −1 week, and in 11 of 13 cases at the occurrence of decelerations.

Comment

The results of this longitudinal study demonstrate that in growth-retarded fetuses, who are eventually delivered because of repetitive FHR decelerations, there is a gradual decline in long-term FHR variation. However, variation often remains within the normal range (> 30 milliseconds) until (or even after) late decelerations occur. This finding is in agreement with data from earlier cross-sectional studies[1, 2] and implies that often the FHR variation of fetuses with IUGR cannot be distinguished from that of normal fetuses before late decelerations appear. Furthermore, the coincidence of the decline in FHR variation and the occurrence of late decelerations indicates that both parameters should be considered as a rather late sign of fetal impairment, because antepartum FHR decelerations are associated with adverse neonatal neurologic outcome.[10]

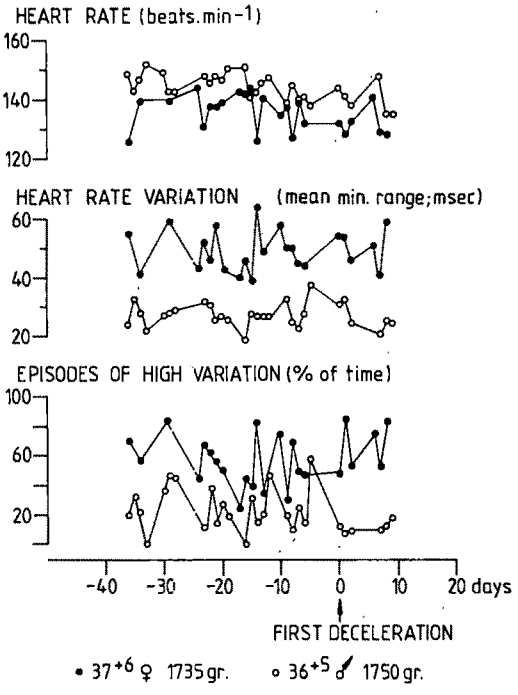


Fig. 4. Basal fetal heart rate, heart rate variation, and occurrence of episodes of high variation in two fetuses with similar birth weights who were delivered at 36 and 37 weeks' gestation, respectively. Data are grouped according to first occurrence of late decelerations. Note interfetal differences.

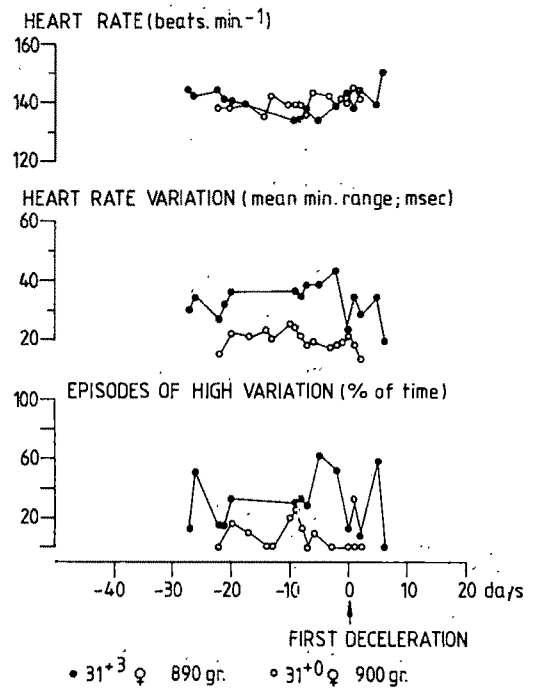


Fig. 5. Basal fetal heart rate, heart rate variation, and occurrence of episodes of high variation in two fetuses with similar birth weights who were delivered at 31 weeks' gestation. Data are grouped according to first occurrence of late decelerations.

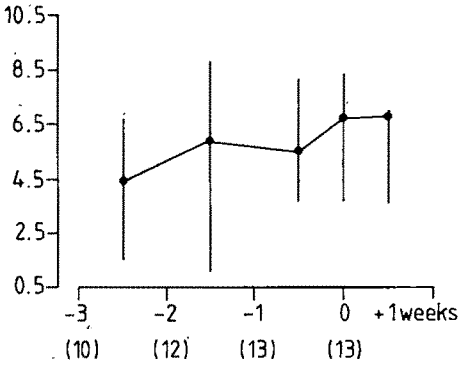


Fig. 6. Changes with time in pulsatility index of umbilical artery in 13 growth-retarded fetuses. Pulsatility index decreases with gestation; therefore data are expressed as number of standard deviations by which measured values differed from appropriate normal mean for gestation. Data are grouped according to first occurrence of late decelerations and are presented as medians and interquartile ranges. Numbers in parentheses are number of fetuses included.

In the rhesus monkey it has been found that with progressive deterioration of the fetal condition, late heart rate decelerations first appeared.[11] This condition was associated with hypoxemia. Heart rate variation (accelerations) decreased later, when the arterial pH also fell. In the human fetus decreased FHR variation occurs (on average) when decelerations appear. Decelerations are, however, dependent on the amount of uterine activity (frequency, duration, and intensity of Braxton Hicks contractions), and with recordings of longer duration or with induced contractions a different time relationship may be found. In this respect it should be noted that in our study decelerations were only present in about 40% of records made after the occurrence of the first deceleration.

It is likely that the initial reduction in FHR variation is mediated by fetal hypoxemia and that evident acidemia is absent until terminal FHR patterns develop. Evidence for this assumption has been provided by reports on associations between decreased FHR variation or development of antepartum FHR decelerations and fetal hypoxemia diagnosed at cordocentesis or at elective cesarean section.[2-4, 12] In addition, maternal hyperoxygenation in pregnancies complicated by IUGR has demonstrated that improved fetal oxygenation results in an increase of FHR variation,[13] whereas variation is temporarily decreased directly after "hypoxemic" events (late decelerations).[14] In fetal acidemia more severe reductions in FHR variation have been described, with simultaneous (shallow) decelerations following each Braxton Hicks contraction (terminal FHR pattern).[4-6]

In this study the interval between the last computerized FHR record and cesarean section varied considerably (1 to 48 hours). The correlation coefficient between FHR variation of the last records and umbilical arterial Po_2 at cesarean section was, however, in the same range ($r = 0.56$, $n = 9$; not significant; see Table I) as described by others ($r = 0.52$ and $r = 0.66$, respectively).[3, 12] In our study and in that of Smith et al.[3] FHR variation was not significantly correlated with pH at cesarean section. In a series including severely compromised fetuses, a significant correlation between mean 1-minute range and pH at cordocentesis has been described ($r = 0.69$).[12]

Although the evolution of the FHR patterns was similar in most fetuses, in some cases decelerations were preceded by reduced FHR variation for up to 5 weeks; in others the variation remained within the normal range even in the presence of decelerations. Differences in the trends in FHR changes could not be explained by differences in head/abdomen circumference ratios, birth weight, or abnormalities in Doppler velocity waveforms. However, they might reflect variations in adaptation to chronic compromise. Further evidence for adaptation to an adverse intrauterine environment can be derived from the finding that the development of decelerations coincided with a small but significant increase in basal FHR, a finding also reported in a previous cross-sectional study.[2]

However, perhaps prolonged low FHR variation in the absence of decelerations should be interpreted as

evidence for an inherently low FHR variation.[15] In normal fetuses there are large interindividual differences in FHR variation; within the same individual variation remains within a much narrower range.[15, 16] The data of this study indicate that this premise also applies to fetuses with IUGR (Figs. 4 and 5). In spite of interindividual differences, most fetuses had a progressive decline in FHR variation. Thus, in the surveillance of the fetus with IUGR, it is better to use each fetus as its own control.[17] This is facilitated by an objective numeric analysis of FHR variation.

In fetuses with IUGR, changes in umbilical artery waveforms usually precede FHR decelerations. In studies in which the latter were taken as an end point, abnormal waveforms had been present for at least 9 days[18] or had been present 17 days on average.[19] A study from our institute showed that the duration of this interval differs considerably (0 to 60 days) and that FHR decelerations occasionally occur when Doppler recordings are still normal.[19] In the present study there were two cases with normal waveforms at the occurrence of decelerations and one with a normal pattern still present just before delivery. To a large extent the considerable differences in time interval between first abnormal waveform and occurrence of FHR abnormalities can be explained by differences in gestational age. In early pregnancy this interval appears to be much longer than it is at late gestation.[19] The variable interval between the first abnormal Doppler recording and abnormality in the FHR indicates, however, that Doppler recordings of the umbilical artery are of limited value with respect to the timing of delivery and that a normal umbilical artery pusatility index cannot be considered a definite proof of fetal well-being.

In summary, with progressive deterioration of the condition of the fetus with IUGR, there is a gradual fall in heart rate variation. Both antepartum decelerations and/or reduceed FHR variation are associated with fetal hypoxemia and/or acidemia. The main additional values of numeric analysis of FHR variation are the identification of those fetuses, in whom reduced variation proceeds the development of decelerations and the provision of objective and longitudinal data on progressive deterioration in fetuses with apparently normal FHR variation or in fetuses with abnormal patterns in whom further maturation in utero is considered.

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Eosinophilic cystitis during pregnancy

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The first report of a case of eosinophilic cystitis during pregnancy is presented. Premature cervical dilatation was associated with onset of the disease at 19 weeks' gestation. (AM J OBSTET GYNECOL 1992;166:28-9.)

Key words: Eosinophilic cystitis

A case of eosinophilic cystitis during pregnancy is described.

Case report

A 31-year-old, gravida 3, para 2 woman had midline pelvic cramps associated with intermittent loss of a small amount of clear fluid at 19 weeks' gestation. Speculum examination, Nitrazine test, and fern test results were negative. She ambulated with Nitrazine paper on a perineal pad until an episode of cramping occurred that yielded a positive test result. Ultrasonographic examination revealed a normal amount of amniotic fluid, and amniocentesis with instillation of indigo carmine dye ruled out ruptured membranes. However, funneling of the membranes was noted, and digital examination revealed cervical dilatation of 1.5 cm with prolapsed membranes into the endocervical canal. Uterine monitoring did not reveal activity. Urinalysis revealed 5 to 10 red blood cells per high-power field, and a complete blood cell count was normal without eosinophilia.

Her medical history included bronchial asthma, genital herpes, and recurrent urinary tract infections for which she underwent cystoscopic examination with normal findings at age 21 years. Obstetric history was unremarkable except for postpartum *Campylobacter* enteritis after her first delivery. During the index pregnancy she received albuterol inhaler treatments beginning at 7 weeks, miconazole vaginal cream at 11 weeks, and prenatal vitamin supplementation. Genetic amniocentesis performed because of a low maternal serum α-fetoprotein level, revealed a normal 46,XX karyotype.

After preparation of the vulva and vagina with povidone-iodine solution, a McDonald cerclage was placed. Saline solution was instilled into the bladder to assist in reduction of the prolapsed membranes. She received prophylactic tocolysis in the form of terbutaline 2.5 mg orally every 6 hours for 48 hours. She had postoperative urinary spasms with involuntary voiding around the urinary catheter. This did not improve with catheter change, and repeat urinalysis results revealed innumerable red blood cells and a few bacteria. Ampicillin 2 gm was given intravenously every 6 hours for presumed severe cystitis; subsequently 1 gm cefoperazone every 12 hours was added. Because her symptoms did not improve, she had a cystoscopic examination with biopsy revealing marked edema, vascular congestion, lymphangiectasia, and perivascular eosinophilic infiltration consistent with eosinophilic cystitis (Fig. 1). Urine cultures for bacteria, *Chlamydia*, herpes simplex, and tuberculosis were negative. Some improvement was obtained with diphenhydramine 50 mg orally every 8 hours and phenazopyridine 200 mg every 12 hours. She declined a trial of steroids. Her symptoms markedly improved by 23 weeks' gestation but recurred between 24 and 34 weeks. Fern testing was done at each visit and with slides mailed in by the patient three times a week. At 31 weeks, persistent diarrhea developed, sigmoidoscopic examination was normal, and the diarrhea gradually resolved with a lactose-reduced diet. Because of the unknown fetal effects of eosinophilic cystitis, antepartum surveillance in the form of weekly nonstress tests and serial ultrasonography was done. At 36 weeks, external cephalic version was performed for breech presentation. At 39 weeks, ultrasonographic examination showed the fetal weight (3651 gm) to be consistent with a large-for-gestational-age fetus with the Lubchenko curve.[1] The patient had a favorable Bishop score of 7; labor was induced, and the patient was delivered of an 8 pound 4 ounce (3737 gm), large-for-gestational-age female infant with Apgar scores of 8 at 1 and 9 at 5 minutes. Umbilical artery pH was 7.42. Placental weight was 680 gm; there was no evidence of eosinophilic inflammation. The postpartum course was normal; urinalysis results were normal >5 months since delivery.

Comment

In 1988 Castillo et al.[2] reviewed the literature, which contained 42 cases of eosinophilic cystitis since the initial report in 1960.[2] None of the cases involved pregnancy. Although parasites were proved to be the cause in some cases, allergy is the most commonly incrimi-

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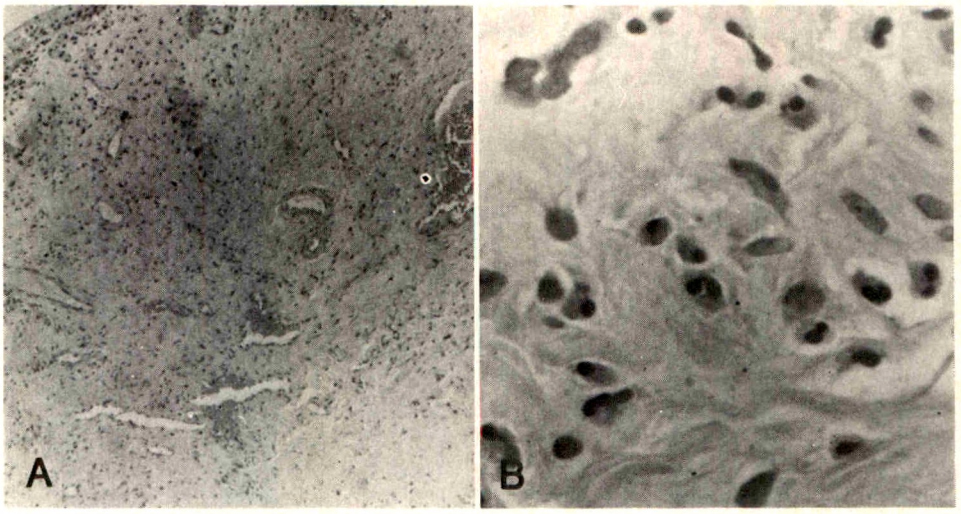


Fig. 1. Low **(A)**– and high **(B)**–magnification photomicrographs of bladder mucosa reveal marked edema, vascular congestion, lymphangiectasia, and perivascular eosinophilic infiltrate consistent with eosinophilic cystitis. (**A,** ×4. **B,** ×40.)

nated factor. The spectrum of disease ranges from mild cystitis to a chronic, relentless cystitis with upper tract damage. Severe blood loss has also occurred. The most common finding is a small-capacity bladder. Urine cultures are sterile in 70%. Treatment regimens include antihistamines, intravesical dimethylsulfoxide, and steroids. Cystectomy and urinary diversion have been required in severe cases. This case of eosinophilic cystitis in pregnancy presented similar to premature rupture of membranes and preterm labor. However, amniocentesis and uterine monitoring ruled out these conditions. The positive Nitrazine test was likely the result of hematuria; the abdominal cramps were believed to originate from the urinary bladder. It is possible that the povidone-iodine vaginal preparation may have entered the bladder through catheterization and served as a potential allergen. Instillation of the saline solution into the bladder may have also aggravated the condition. However, the onset of the symptoms clearly antedated these procedures. Although cervical cerclage was required, further reports will be necessary to determine whether incompetent cervix is a frequent association with eosinophilic cystitis in pregnancy.

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Upper vaginectomy for in situ and occult, superficially invasive carcinoma of the vagina

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Between Aug. 1, 1985, and July 31, 1990, 32 patients underwent upper vaginectomy for grade 3 vaginal intraepithelial neoplasia. Thirty-one of these patients had undergone hysterectomy, 25 because of cervical neoplasia. Fourteen patients had undergone treatment for vaginal intraepithelial neoplasia. Nine (28%) had invasive cancer on final pathologic examination. Among the remaining 23 patients, recurrence of vaginal neoplasia developed in four (17%), with a mean time to recurrence of 78 weeks, and one was found to have superficial invasion at the time of recurrence. The remaining 19 patients remain alive with no evidence of recurrent disease at a mean follow-up interval of 152 weeks. In our patients upper vaginectomy was efficacious for the diagnosis of occult invasive carcinoma of the vagina and for the treatment of in situ and superficially invasive carcinoma of the vagina. (AM J OBSTET GYNECOL 1992;166:30-3.)

Key words: Upper vaginectomy, vaginal intraepithelial neoplasia

Vaginal intraepithelial neoplasia accounts for approximately 0.4% of lower genital tract intraepithelial neoplasia.[1] Identifiable risk factors for this condition include cervical intraepithelial neoplasia, human papillomavirus infection, immunosuppression, and possibly previous pelvic radiotherapy.[2-5] The invasive potential of vaginal intraepithelial neoplasia appears to be similar to that of cervical intraepithelial neoplasia.[2, 6] The process most commonly occurs in the upper third of the vagina and frequently is multifocal.[3-11] Vaginal intraepithelial neoplasia is usually diagnosed during an investigation of an abnormal Papanicolaou smear; it is not grossly visible and does not usually produce symptoms. The diagnosis is usually established at colposcopy with directed biopsy. A wide variety of treatments have been used, most commonly consisting of local excision, partial or total vaginectomy, radiotherapy, laser vaporization or topical 5-fluorouracil administration.[12] Each of these treatment methods has advantages and disadvantages, and none has proved superior efficacy over the others. This is a report of our experience with the use of upper vaginectomy for grade 3 vaginal intraepithelial neoplasia.

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Material and methods

The charts of all patients with grade 3 vaginal intraepithelial neoplasia managed by our division between Aug. 1, 1985, and July 31, 1990, were reviewed retrospectively. A total of 45 patients were identified. Of these, 32 were treated either primarily or secondarily with upper vaginectomy. It was not possible to define accurately how patients were selected for upper vaginectomy over other forms of treatment. In general, upper vaginectomy was performed for grade 3 vaginal intraepithelial neoplasia when the lesion was confined to the upper one third to upper one half of the vagina, especially when invasive cancer was suspected or could not be adequately ruled out by colposcopically directed biopsy. All upper vaginectomies were performed by an obstetrics and gynecology resident or fellow under the direct supervision of one of the gynecologic oncology attending physicians. All procedures were performed in the operating room with the patient under general or regional anesthesia. An attempt was made to include a 1 cm margin of surrounding normal-appearing mucosa. Patients were generally admitted on the day of surgery and kept overnight in the hospital with a vaginal pack in place. Patients were examined at 2 weeks, 8 weeks, and then every 2 to 3 months after surgery for the first 2 years and then every 6 months thereafter, unless there was recurrence of the disease. Five of the patients included in this study were also the subject of a prior report.[13]

Results

The mean age of the 32 study patients was 64 years, with a range of 36 to 85 years. Thirty-one (97%) pa-

tients had undergone hysterectomy. Twenty-five of these were for squamous cervical neoplasia, four were for benign disease, and two were for unknown reasons. Five patients had undergone pelvic radiotherapy for malignancy. Fourteen patients (44%) had undergone therapy for vaginal intraepithelial neoplasia or vaginal human papillomavirus infection. These treatments included one or two laser vaporizations in eight patients, upper vaginectomy in one patient, 5-fluorouracil administration in two patients, and multiple types of treatment in three patients.

The vaginal intraepithelial neoplasia was located in the apex or upper one third of the vagina in 23 patients, it was multifocal in the upper one half of the vagina in five patients, and it was in a vaginal pocket or inclusion cyst in four patients. The area of the vagina encompassed by the lesions ranged from 1 × 1 to 3 × 5 cm. The mean operative time was 60.5 minutes with a range of 20 to 105 minutes. The mean estimated blood loss was 187 ml with a range of 50 to 1150 ml. The patient who lost 1150 ml had hypersplenism-related thrombocytopenia, and focal, superficially invasive cancer was found on the final pathology specimen. Including this patient, three patients had an intraoperative estimated blood loss of ≥500 ml. Other intraoperative complications included premature ventricular contractions in one patient, which were successfully treated with intravenous lidocaine, and a cystotomy in another patient, which was immediately repaired and healed uneventfully. In one patient severe bleeding estimated to be 1000 ml developed approximately 3 weeks postoperatively. This was successfully managed with vaginal packing containing ferric subsulfate solution. Febrile morbidity developed in one patient and was believed to be related to operative site cellulitis, which responded well to intravenous antibiotics. There were no other postoperative complications.

Of the 32 patients, nine had invasive squamous cell carcinoma in the upper vaginectomy specimen. In all of these patients the lesion was at the vaginal apex in the region of the vaginal cuff scar. Four had undergone hysterectomy for grade 3 cervical intraepithelial neoplasia. Three had undergone radical hysterectomy for invasive squamous cell carcinoma of the cervix, and two had undergone radiotherapy (one with hysterectomy).

Four of the nine were frankly invasive (from 3.5 mm to full-thickness involvement) and were treated subsequently with radiotherapy. One of these four subsequently died of carcinoma 23 months later. The remaining three patients remained alive with no evidence of recurrent disease at 37, 42, and 46 months after radiotherapy.

The other five patients had invasion measuring <2 mm in depth. In one of these patients who was severely immunocompromised, several foci of recurrent, superficially invasive (<2 mm) squamous cell carcinoma developed in the vagina 31 months after upper vaginectomy; she was treated with radiotherapy, which was completed 2 months before completion of this study. The other four with superficially invasive carcinoma of the vagina have received no further treatment and remain alive with no evidence of recurrent disease at 4, 31, 44, and 54 months after upper vaginectomy.

The 23 remaining patients all had grade 3 vaginal intraepithelial neoplasia on the final pathologic examination of the upper vaginectomy specimen. Margins were clear on all but one patient. She received no further treatment and remains without evidence of recurrence at 6 months. Of the 23 patients, 19 (83%) remain without evidence of recurrence at a mean follow-up of 38 months, with a range of 6 to 73 months. In four patients recurrence of vaginal neoplasia developed, with a mean time to recurrence of 19 months and a range of 3 to 32 months. Recurrence developed in one of these patients 64 weeks after upper vaginectomy, and superficially invasive cancer <1 mm was found on pathologic examination of a repeat upper vaginectomy specimen. She remains without evidence of recurrence 7 months later. Recurrence was managed by repeat upper vaginectomy in two of the other patients and simple excision in the office in the fourth patient. Follow-up information regarding anatomy and sexual function was inadequate for reporting. However, of the 15 patients treated with upper vaginectomy only and with no history of radiotherapy, five had a vagina obviously adequate for intercourse and five had an obviously shortened vagina. Such information was not recorded for the other five patients.

Comment

A wide variety of methods have been used to treat vaginal intraepithelial neoplasia, including partial to total vaginal excision, radiotherapy, laser vaporization, and topical 5-fluorouracil.[2, 7, 9, 12-24] In general, good results have been obtained with these methods, although the results have been more variable with laser vaporization.[12, 14-17, 19-24] Laser vaporization for vaginal intraepithelial neoplasia has been variously reported to have a 50% to 100% success rate. Some of these studies included patients who had undergone two laser vaporizations. Partial or total vaginectomy has been considered by many to be the treatment of choice, mainly because it excises the abnormal tissue and provides a specimen for complete histologic review.[13, 18, 19, 25, 26] One of the main detractions of partial vaginectomy is shortening or stenosis of the vagina. Preventive measures include not closing the mucosa and using a dilator with estrogen vaginal cream in the early postoperative period. Covering the excised area with a skin graft is another useful alternative.[2] Several advantages of

laser vaporization and 5-fluorouracil have been cited, including reduced operative morbidity, less operative time, and improved subsequent sexual function.[14-18, 20-23, 26] Laser vaporization or 5-fluorouracil may also be preferable in previously irradiated patients because of the excessive morbidity associated with vaginectomy in these women.

Before treatment with laser vaporization is considered, an accurate appraisal of the extent and severity of the disease must be made. This is facilitated by using colposcopy, Lugol's iodine, palpation, and multiple biopsies. Laser vaporization should not be performed unless the full extent of the abnormal vaginal epithelium can be visualized, there is no suspicion of an invasive process, and there is no gross scarring or distortion of the posthysterectomy vaginal cuff. The last point has been made by several authors, including a prior study from this institution in which some of the patients from the current study are included.[13-21] The results of the current study underlines the problem of occult invasive vaginal carcinoma discovered in the patient diagnosed as having grade 3 vaginal intraepithelial neoplasia. Nine of our 32 patients (28%) had invasive carcinoma on the upper vaginectomy specimen. Any treatment other than excision of this area with histologic evaluation would have led to inappropriate treatment. In all nine of these patients the lesion was at the vaginal apex in the region of the vaginal cuff scar. Vaginal intraepithelial neoplasia in the upper vagina of women who have undergone hysterectomy probably should be excised along with the vaginal cuff scar. This seems especially important for patients whose hysterectomies were performed because of cervical cancer or intraepithelial neoplasia, whose vaginal cuffs are prominent or distorted, or who have treatment failure with laser vaporization or topical 5-fluorouracil. All such patients should undergo a careful bimanual rectovaginal examination to detect the presence of obvious induration or nodularity of the vaginal cuff.

None of the patients in the present study had a preoperative diagnosis of invasive cancer. The standard treatment for invasive carcinoma of the vagina is radiotherapy.[27] On review of the upper vaginectomy specimens of five of the patients, superficial invasion <2 mm was found and was believed to be well cleared. It was thought that these patients received adequate treatment and radiotherapy was not administered. As noted in Results, one of the patients did have recurrent superficially invasive disease in the vagina and was treated with radiotherapy. Other small studies have supported the use of partial or total vaginectomy for the treatment of superficially invasive (<2.5 mm) squamous cell carcinoma of the vagina.[28, 29]

On the basis of the results of this study, we believe that upper vaginectomy is the treatment of choice for grade 3 vaginal intraepithelial neoplasia diagnosed at the vaginal apex in the region of a vaginal cuff scar. Whether upper vaginectomy is efficacious therapy for superficially invasive carcinoma of the vagina will require further study. In the presence of multifocal or extensive grade 3 vaginal intraepithelial neoplasia involving large areas of vaginal mucosa, laser vaporization of the disease away from the vaginal apex could easily be combined with an excision of the vaginal apex and cuff scar area.

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Transient symptomatic subendocardial ischemia during intravenous magnesium sulfate tocolytic therapy

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Although β-sympathomimetic tocolytic therapy has been associated with transient subendocardial ischemia, magnesium sulfate has rarely been noted to cause acute chest pain and is, in fact, known to improve myocardial perfusion in patients with variant angina. We believe this report represents the first case in which intravenous magnesium sulfate administered as a tocolytic agent caused acute chest pain accompanied by transient electrocardiographic evidence of subendocardial ischemia. (Am J Obstet Gynecol 1992;166:33-5.)

Key words: Preterm labor, magnesium sulfate, myocardial ischemia

Magnesium sulfate is a successful, inexpensive, and relatively nontoxic tocolytic agent with few side effects. The exact mechanism of its tocolytic activity is unclear. The incidence of acute chest pain in association with this therapy is low and has been reported at 1.1%. We report the first documentation of acute chest pain accompanied by electrocardiographic signs of transient subendocardial ischemia occurring in conjunction with this regimen.

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6/1/31815

Case report

A 29-year-old Oriental woman, gravida 1, para 0, was admitted to labor and delivery at Strong Memorial Hospital because of preterm labor at 35 weeks 1 day of gestation. Her medical history was unremarkable, and her pregnancy was uneventful until she was seen on this occasion. Physical examination 4 hours before her referral disclosed the uterine cervix to be 90% effaced and dilated to 1 cm. On admission, she was noted to be in good general health. Blood pressure was 110/65 mm Hg. The cervix was 90% effaced and dilated to 2 to 3 cm with the vertex presenting at S − 3 station. Although she initially responded to intravenous hydration, the uterine contractions recurred, and intravenous magnesium sulfate was administered at a rate of 3 gm/hr after a loading dose of 4 gm. Ten hours after this treatment was initiated, the patient began complaining of nonradiating substernal chest pain. No respiratory distress was noted, and blood pressure was

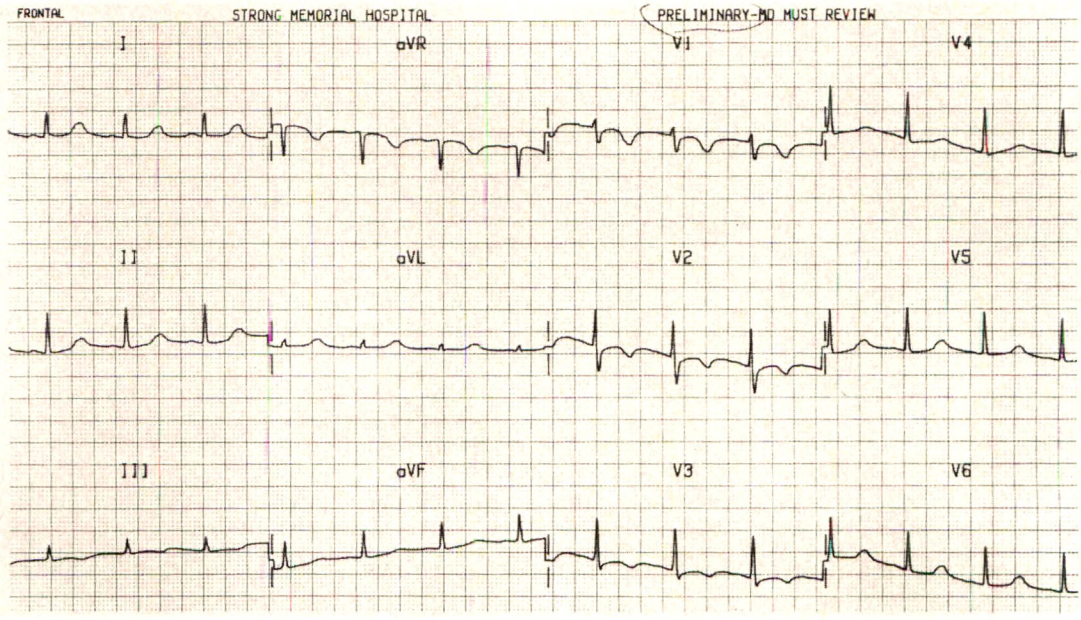


Fig. 1. Electrocardiogram during acute chest pain. Note inverted T waves in leads L_3 and V_1 through V_3 (speed 25 cm/sec, voltage 1 cm = 1 mV).

80/50 mm Hg with a heart rate of 100 beats/min. Electrocardiographic examination revealed a normal sinus rhythm at 84 beats/min with inverted T waves in L_3 and V_1 through V_3 leads (Fig. 1). Chest roentgenogram was normal. Laboratory evaluation revealed hemoglobin 12.2 gm, hematocrit 36%, sodium 135 mEq/L, potassium 3.9 mEq/L, glucose 93 mg/dl, creatine kinase 114 mU/ml (normal 0 to 122), MB (myocardial component) isozyme fraction 5% (normal). Serum magnesium level was 6.3 mEq/L. The intravenous magnesium sulfate was decreased to a rate of 2 gm/hr with resolution of chest pain, although a repeat electrocardiogram continued to demonstrate the inverted T waves. After 30 hours magnesium sulfate was gradually tapered successfully and discontinued with resolution of the electrocardiographic changes. The patient was discharged on day 3 after a normal echocardiogram. At 41 weeks 3 days' gestation, because of an arrest of the second stage of labor, a vacuum extraction was performed, with the delivery of a male infant weighing 3240 gm with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively.

Comment

β-Sympathomimetic therapy for preterm labor has been associated with symptomatic and asymptomatic myocardial ischemia that appears to be localized in the subendocardial region. Subendocardial oxygen supply may be compromised during β-sympathomimetic administration. This is thought to be a result of induced tachycardia resulting in a shortened diastole, i.e., the time of myocardial perfusion, and the placenta's effect on peripheral vascular resistance with diminished aortic diastolic blood pressure. In many centers magnesium sulfate has replaced β-sympathomimetic therapy as the first-line drug for tocolysis of preterm labor. To date, there have been no reported cases of myocardial ischemia during magnesium sulfate administration, when it was used for this purpose.

Intravenous magnesium sulfate is known to have antiarrhythmic effects and has, in fact, been used as a possible protective agent in patients with acute myocardial infarction, reducing conduction disturbances and in-hospital mortality in these patients. Furthermore, intravenous magnesium sulfate suppresses exercise-induced angina.[1] The size of the myocardial perfusion defect as measured by thallium 201 scintigraphy was significantly less in 13 patients with variant angina receiving magnesium sulfate than in 11 control patients receiving placebo.[1] The beneficial effect of magnesium in patients with variant angina is thought most likely to represent improvement of regional myocardial blood flow by suppression of coronary artery spasm. It is therefore of significance that acute chest pain with transient myocardial ischemia and associated electrocardiographic changes occurred during administration of this drug for tocolysis of preterm labor.

This event may have resulted from hypotension as a secondary effect of intravenous magnesium sulfate, compounded by a physiologic reduction in diastolic blood pressure normally occurring in pregnancy, which led to a transient but substantial decrease in subendocardial perfusion. Dandavino et al.[2] have previously shown that bolus administration of magnesium sulfate to control levels occurred within 5 minutes of bolus injection and no change in blood pressure occurred

during subsequent infusion of magnesium sulfate at 2 or 4 gm/hr. In our case symptoms and associated electrocardiographic changes were noted 10 hours into treatment. The mechanism of this drug-related sequela remains to be deciphered. Nevertheless, because of the widespread use of magnesium sulfate in pregnancy, this case is reported to bring attention to this seemingly rare drug event.

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Possible transplacental transmission of human papillomaviruses

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OBJECTIVE: The objective of this study was to examine the possibility of intrauterine human papillomavirus infection of fetuses by transplacental transmission of human papillomavirus before delivery.
STUDY DESIGN: Specimens of cervicovaginal cells and peripheral blood mononuclear cells were obtained from 52 consecutive pregnant women in the third trimester of pregnancy. Cord blood specimens were also obtained from the neonates born to these mothers. Presence of human papillomavirus types 16 and 18 deoxyribonucleic acid was analyzed by an in vitro enzymatic deoxyribonucleic acid amplification method.
RESULTS: Human papillomavirus type 16 deoxyribonucleic acid was found in 6 (11.5%) cervicovaginal and in 9 (17.3%) peripheral blood mononuclear cell specimens. Seven cord blood specimens from neonates born to mothers who were positive for peripheral blood mononuclear cell human papillomavirus type 16 deoxyribonucleic acid were found to contain human papillomavirus type 16 deoxyribonucleic acid. One cervicovaginal and two peripheral blood mononuclear cell specimens contained human papillomavirus type 18 deoxyribonucleic acid, but none of the cord blood specimens contained human papillomavirus type 18 deoxyribonucleic acid.
CONCLUSION: These results seem to suggest possible transplacental transmission of the virus and the potential association of such transmission with the status of human papillomavirus in peripheral blood mononuclear cells. (Am J Obstet Gynecol 1992;166:35-40.)

Key words: Human papillomavirus, transplacental transmission, polymerase chain reaction (PCR), peripheral blood mononuclear cells

Depending on the detection method used and the type of tissue examined, between 10% and 60% of clinically and histologically normal cervical specimens from a general population were found to contain human papillomavirus (HPV) deoxyribonucleic acid (DNA).[1-5] Although most of these infections are asymptomatic and subclinical in nature and the clinical and biologic significance of these findings is not clear, the prevalence of HPV even among the general population is probably quite high. Management of pregnant patients with HPV infections takes on additional importance because of the possibility of maternal-fetal transmission. The issue of possible maternal-fetal HPV transmission is a pressing concern for pregnant patients because several reports have suggested that the incidence of genital HPV infection is higher in pregnant women than in nonpregnant women.[6-9] Given that the behavior of urogenital human papillomavirus infections during pregnancy is not clear and that the exact mode and risk of the maternal-fetal HPV transmission in clinically

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asymptomatic patients are almost completely unknown, it is tempting to speculate that inoculations may occur during passage of the fetus through the birth canal. On the other hand, reports of juvenile laryngeal papillomatosis,[10] congenital condyloma acuminatum,[11] and the presence of HPV DNA in the foreskin,[12] oral cavity, and amniotic fluid[13] of clinically normal neonates delivered by cesarean section suggest the likelihood of intrauterine HPV infection by transplacental transmission before delivery.

We report, in the following text, the results of using an extremely sensitive and specific method of polymerase chain reaction to assess the presence of HPV type 16 and 18 DNA sequences in cord blood of neonates born to women with urogenital or peripheral blood mononuclear cell HPV infection.

Material and methods

Experimental protocol. Blood specimens were obtained from 52 consecutive pregnant women in the third trimester of pregnancy who were receiving routine antepartum care at Chang Gung Memorial Hospital in Taipei, Taiwan. Cervicovaginal cells were also collected from these women at the same time by cervical lavage with 2 to 4 ml of phosphate-buffered saline solution (20 mmol/L, pH 7.0) containing sucrose (200 mmol/L), streptomycin 50 μg/ml), vancomycin (100 μg/ml), and nystatin (25 U/ml). None of the patients had symptomatic or known urogenital HPV infections at the time of sampling. The average age of the patients was 28.5 years (median 28 years). Thirty-two and 14 of the women were pregnant for the first and second times, respectively, and the rest of them were pregnant for the third time or more. Informed consents were obtained from all participants to obtain blood from them and their infants.

Polymerase chain reaction amplification of HPV DNA. The procedures for the amplification and subsequent detection of HPV type 16 and 18 DNA in clinical specimens were those we have described earlier[14, 15] with the following modifications to incorporate an additional round of "nested" amplification. The nested polymerase chain reaction requires a second or inner set of primers that locate inside the initial or outer primers and uses the resulting products from the initial amplification as a template for amplification. The nested amplification both increases the overall sensitivity and enhances the specificity of polymerase chain reaction by minimizing false-positive results.[16, 17] For the nested amplification, 2 μl of the reaction mixture from the initial amplification reaction was amplified with same procedures in a freshly prepared 100 μl reaction mixture containing the same reaction components as the initial polymerase chain reaction and the new set of nested primers. The genomic region within the HPV E6 genes was chosen to amplify because this region of HPV genome is almost always retained in the HPV DNA integrated in cancer tissues.[18] The sequences of the antisense and sense strands of primers that flank the 445 base pair E6 gene of HPV type 16 DNA in the first round of amplification are CGTGTTCTTGATGATCTGC and GAACTGCAATGTTTCAGGACC (the 5′-end bases of these two primers are 540nt and 96nt, respectively on the HPV type 16 genome). The sequences of the corresponding nested primers that cover 422 base pairs of DNA on the HPV type 16 genome are GCAACAAGACATACATCGACCG (523nt) and CAATGTTTCAGGACCCACAGG (102nt). The first and nested sets of primers for HPV type 18 DNA amplification are CTCGTGACATAGAAGGTCAACCGG (676nt) and CACACCACAATACTATGGCGCGCT (91nt) and CAATGTTGCCTTAGGTCCATGC (613nt) and CTACAAGCTACCTGATCTGTGC (137nt), respectively. The sizes of the DNA flanking by the first and nested sets of primers used in this study for the HPV type 18 DNA are 586 and 477 base pairs, respectively. Additional primers complementary to HPV E7 and L1 genes are also used.

Establishment of HPV positivity. After the DNA amplification, one-tenth of the amplification reaction mixture was separated by electrophoresis in 2% agarose gel containing 0.5 μg/ml ethidium bromide and visually inspected under ultraviolet light for the presence of 422 and 477 base pair DNA bands to determine the HPV type 16 and 18 positivity, respectively. Authenticity of this amplified DNA was confirmed by restriction endonuclease analysis and by Southern blot DNA hybridization with an internal oligonucleotide probe.[15, 19] The sequences of oligonucleotide probes are AGCTGCAAACAACTATACATGATATAATATTAGAATGTGT (for type 16) and AACTGAACACTTCACTGCAAGACATAGAAATAACCTGTGT (for type 18). The oligonucleotide probes were labeled at their 5′-end with γ–phosphorus 32–adenosine triphosphate by polynucleotide kinase to approximately 5×10^6 cpm/pmol. Procedures for Southern blot hybridization have been described previously by others[19] and were modified for oligonucleotide probes as follows: After hybridization, the filters were washed sequentially in 2× SSC (1× SSC is 0.1 mol/L sodium chloride and 0.015 mol/L sodium citrate) and 0.1% sodium dodecyl sulfate twice for 10 minutes each at room temperature and then in 1× SSC and 0.1% sodium dodecyl sulfate three times for 20 minutes each at 55° C. Restriction endonucleases were purchased from either Boehringer Mannheim (Mannheim, Germany) or BRL (Gaithersburg, Md.) and used according to manufacturer's recommendations.

Precautions against polymerase chain reaction contamination and false-positive results. Because of the extraordinary power of the amplification procedures, precautions were taken to minimize or eliminate the possibility of contamination during both sampling and subsequent processing. These precautions included ex-

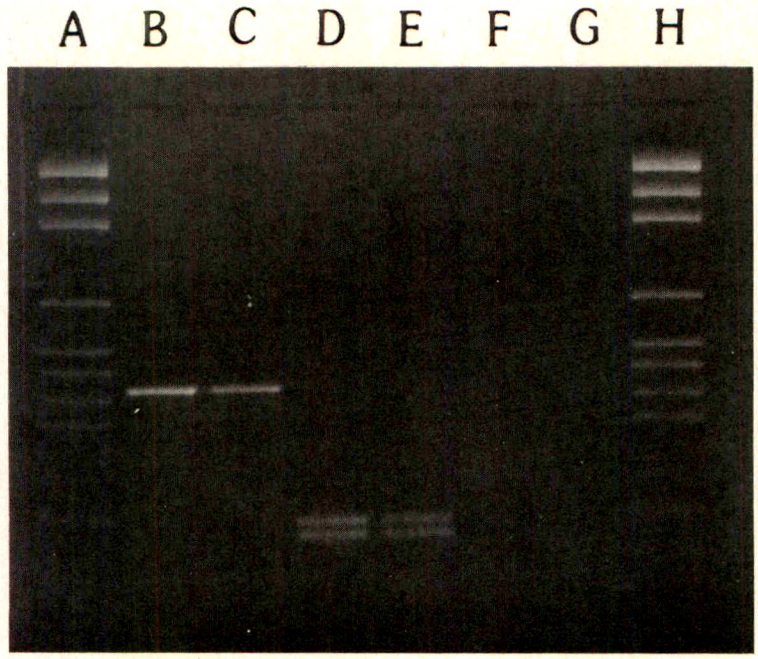


Fig. 1. Agarose gel electrophoresis of amplified HPV type 16 DNA. pGEM-3 DNA digested with a mixture of restriction endonucleases *Hin*fI, *Rsa* I, and *Sin* I was used as size standards in *lanes A* and *H*, and the sizes are (from top to bottom) 2645, 1605, 1198, 676, 517, 460, 396, 350, 222, 179, 126, 75, 65, 51, and 36 base pairs. *Lanes B* and *C* are amplifications of DNA prepared from cervicovaginal cells of a HPV type 16–infected patient and from CaSki cells, respectively. *Lanes D* and *E* are restriction endonuclease *Dra* I–digested amplified DNA in *lanes B* and *C*, respectively. *Lanes F* and *G* are respective amplifications of 50 ng of human DNA and 1 ng of bacterial *E. coli* DNA with HPV type 16 primers.

tensive cleaning of the skin area and umbilical cord before the venipuncture and the use of closed and sterile Vacutainer blood collecting tubes (Becton Dickinson, Rutherford, N.J.). Special measures were also taken to minimize sample-to-sample contamination and polymerase chain reaction product carryover to avoid false-positive results. These precautions include aliquoting of all reagents, physical separation of samples before and after polymerase chain reaction, and meticulous laboratory techniques. Furthermore, all reagents were irradiated with ultraviolet light to inactivate any contaminated double-stranded DNA[20] that may have been present before sample DNA was added and the polymerase chain reaction started. Fifth nanograms of human DNA or 1 ng of *Escherichia coli* DNA was used as a negative control, which always yielded negative results. Multiple reagent controls were also included in each polymerase chain reaction assay and gave negative results. Repeated DNA amplification assays of the same specimens at different times produced the same results. Human *Alu* repeated DNA sequences[21] were also amplified with each assay to ensure that enough sample DNA was used in the amplification reaction.

Results

The presence of HPV type 16 DNA in clinical specimens is indicated by the presence of the 422 base pair DNA after two rounds of amplification (Fig. 1, lanes B and C). The lower limit of detection of the amplification used in this study was estimated to be <0.001 pg of HPV type 16 or 18 DNA or approximately 100 HPV viral genomes equivalent. This number was calculated by amplifying serial dilutions of a purified cloned HPV DNA solution with known concentration (data not shown) and by the amplification of HPV type 16 DNA in CaSki cells, which has been suggested to contain about 500 copies of HPV type 16 per cell.[22] This sensitivity of detection is not adversely affected by the presence of as much as 50 ng of human DNA (equivalent to approximately 10,000 cells) or 1 ng of *Escherichia coli* DNA (equivalent to approximately 200,000 bacterial cells). DNA prepared from *Chlamydia trachomatis, Mycoplasma hominis*, Herpes simplex virus types I and II, cytomegalovirus, Epstein-Barr virus, and other types of HPV (including HPV types 1, 6, 8, 11, 18, 31, and 33) does not cross amplify with the HPV type 16 primers used in this study (data not shown).

The specificity of the primers and the authenticity of the amplified 422 base pair HPV type 16 DNA were confirmed by two independent methods. First, the amplified HPV type 16 DNA was treated with a restriction endonuclease with known restriction site on the amplified DNA according to known DNA sequences. When the 422 base pair DNA from amplifying DNA,



Fig. 2. Autoradiograph of Southern blot hybridization test of amplified HPV type 16 DNA with internal oligonucleotide probe. Arrangements of samples are same as in Fig 1. Probe sequence and hybridization procedures have been described in Material and methods. Top bands in *lanes D* and *E* were results of undigested residual 422 base pair DNA. Exposure time was 40 minutes at room temperature without intensifying screen.

Table I. Presence of HPV type 16 DNA in maternal cervicovaginal and peripheral blood mononuclear cells

Neonate's cord blood HPV type 16 DNA	*HPV type 16 DNA in maternal cervicovaginal cells/peripheral blood mononuclear cells*			
	Positive/positive	*Positive/negative*	*Negative/positive*	*Negative/negative*
Positive	1	0	6*	0
Negative	1	4	1	39

*One of the babies was HPV type 16 positive on follow-up when venous blood was assayed for HPV type 16 DNA 3 months after birth.

prepared from either cervicovaginal cells of an infected patient or CaSki cells (which contain HPV type 16 DNA), was treated with restriction endonuclease *Dra* I, the original 422 base pair DNA disappeared and was replaced with new DNA of 220 and 202 base pairs (Fig. 1, lanes D and E). Second, the Southern blot hybridization showed that both the 422 base pair DNA and the 202 base pair restriction endonuclease fragment resulted from *Dra* I digestion hybridized with the oligonucleotide probe (Fig. 2). The specificity of the amplification of HPV type 18 DNA was confirmed by similar procedures. The amplified 477 base pair HPV type 18 DNA could be converted by restriction endonuclease *Xba* I to two DNA fragments of 292 and 185 base pairs, which agreed exactly with the prediction from the known restriction site and HPV type 18 DNA sequences (data not shown). Amplification of HPV types 16 and 18 with primers complementary to E7 and L1 genes yielded the same results as those obtained with primers of E6 genes.

Tables I and II summarize the presence of HPV type 16 and 18 DNA in neonatal cord blood with respect to HPV DNA in maternal cervicovaginal cells and peripheral blood mononuclear cells. HPV type 16 DNA was found in 6 of 52 (11.5%) cervicovaginal cell specimens and in 9 of 52 (17.3%) peripheral blood mononuclear cell specimens from the same group of women. A total of 7 neonates, all born to mothers with peripheral blood mononuclear cell HPV DNA (6 mothers without and 1 mother with cervicovaginal cell HPV DNA), were found to have HPV type 16 DNA in the cord blood collected at birth. On the other hand, cord

Table II. Presence of HPV type 18 DNA in cord blood of neonates

Neonate's cord blood HPV type 18 DNA	*HPV type 18 DNA in maternal cervicovaginal cells/peripheral blood mononuclear cells*			
	Positive/positive	*Positive/negative*	*Negative/positive*	*Negative/negative*
Positive	0	0	0	0
Negative	0	1	2	49

Table III. Presence and type distribution of HPV types 16 and 18 DNA in maternal cervicovaginal and peripheral blood mononuclear cells and in cord blood of neonates

Patient No.	*HPV type 16 DNA*			*HPV type 18 DNA*		
	In maternal cervicovaginal cells	*In maternal peripheral blood mononuclear cells*	*In neonate's cord blood*	*In maternal cervicovaginal cells*	*In maternal peripheral blood mononuclear cells*	*In neonate's cord blood*
1	−	+	−	−	−	−
2	−	+	+	−	−	−
3	−	+	+	−	−	−
6	+	+	−	−	−	−
8	+	−	−	−	−	−
9	−	+	+	−	+	−
10	−	−	−	−	+	−
12	+	−	−	−	−	−
21	+	−	−	−	−	−
25	+	+	+	−	−	−
26	−	+	+	−	−	−
29	−	+	+	−	−	−
37	−	+	+	−	−	−
41	+	−	−	−	−	−
47	−	−	−	+	−	−

Patients 4, 5, 7, 11, 13 to 20, 22 to 24, 27, 28, 30 to 36, 38 to 40, 42 to 46, and 48 to 52 all had negative results.

blood specimens from infants born to two other mothers with peripheral blood mononuclear cell HPV DNA were free of detectable HPV DNA. None of the 39 mothers without cervicovaginal cell and peripheral blood mononuclear cell HPV gave birth to infants who had HPV DNA in cord blood. Follow-up investigation was performed on two babies with cord blood HPV DNA. Venous blood specimens from one of them remained HPV DNA positive 3 months after birth (Table I).

HPV type 18 DNA was found in one cervicovaginal specimen and in two peripheral blood mononuclear cell specimens among the 52 pregnant women. However, none of the cord blood specimens of the neonates born to any of the 52 women was found to contain HPV type 18 DNA, unlike HPV type 16 DNA (Table II).

The presence of HPV DNA and the distribution of HPV types in the maternal cervicovaginal and peripheral blood mononuclear cells and cord blood of neonates born to the 52 pregnant women are summarized in Table III.

Comment

This study seems to have provided information on both the possible transplacental transmission of HPV and the potential association of such transmission with the status of HPV in peripheral blood mononuclear cells.

Although the behavior of HPV in patients with clinically asymptomatic urogenital HPV infections during pregnancy is unclear, our results supported the findings by others, which indicated an increased incidence of genital HPV infection during pregnancy.[6-9] The presence of HPV in both cord and venous blood of infants suggests that the HPV in these infants probably resulted from viral infection before birth through transplacental transmission instead of being acquired from the maternal vaginal secretions at the time of birth. This possibility is supported by the findings that the presence of HPV DNA in cord blood of neonates seems to be more closely related to the status of HPV DNA in maternal peripheral blood mononuclear cells than cervicovaginal cells. Examination of HPV DNA in trophoblastic and villous cells may be able to provide more information on this matter.[23]

We have reported earlier the findings of HPV DNA in peripheral blood mononuclear cells of patients with urogenital HPV infections.[15] We have speculated that HPV in peripheral blood mononuclear cells may serve as a potential source of infection of others or recurrent infection in the same patients and therefore represents route(s) of HPV transmission other than sexual or di-

rect contact.[15] More recently we have succeeded in immortalizing (with Epstein-Barr virus) HPV-infected B lymphocytes from patients, and preliminary results indicated that these B cells produce and secrete HPV virions into culture media (unpublished observations). These findings are consistent with the suggestion that HPV in peripheral blood mononuclear cells may be involved in the possible transplacental transmission of HPV, which may occur during intermittent low-level viremia released from these cells.

The possible association between HPV in peripheral blood mononuclear cells and HPV transmission clearly requires more extensive investigations. Hepatitis type B virus infection of extrahepatic sites, such as peripheral blood mononuclear cells, has been shown to be a major factor in infections of liver grafts.[24] Indeed, a direct transmission of the hepatitis type B virus genome from HBV-positive to HBV-negative peripheral blood mononuclear cells, even in the absence of a detectable hepatitis type B virus virion, has been described.[25]

We believe that the presence of HPV DNA sequences may not in itself indicate the presence of infectious virions, but the findings of HPV DNA in cord blood of neonates born to mothers with HPV in peripheral blood mononuclear cells nevertheless suggest a mechanism by which HPV can be transmitted. Given that HPV growth and host immunologic responses to HPV infection during pregnancy are unclear, longitudinal follow-up investigations on infants with HPV in cord blood is needed for understanding of both the transmission of HPV and any clinical significance it may carry.

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Please see adjoining page for a brief summary of prescribing information.

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BRIEF SUMMARY

Demulen® 1/35: Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients. **Demulen® 1/50:** Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients.

Indication and usage: For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk:* Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen.

Contraindications: Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products.

Warnings:

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. Latter group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myocardial ischemia, occurs with high progestational activity. Consider amount of both steroids in choosing OC. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

Be alert to earliest manifestations of TE disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users. If feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometri cancer with combination or progestogen-only OCs. Studies show no increased breast cancer with OCs or estrogens. Howeve greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens ro and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other ri factors and in various subgroups. Decrease in benign breast tumors in OC users is well known. Some studies suggest increased risk of cervical dysplasia, CIN, erosion, and carcinoma in long-term users. Cervical microglandular hyperplasia h been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OCs. Clos surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunction causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of brea cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasi **4.** *Hepatic adenomas, etc.* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" we associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemoperitoneu in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long-term C users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdominal pai tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Radiolog and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may be usefu Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur with OC us **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring:* Estrogens or progestogens during ear pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may ha increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 3 90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES-expose daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Oth estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL f vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by males. The was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdraw pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascul defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,000 birth compared to 7.8 for those unexposed (P<.05). Female hormone exposure in utero was associated with testicular and ovari cancer. More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were used f threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestoge are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregnant soo post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stoppi OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstrated. Ru out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missi rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withho OCs. If pregnancy is confirmed, stop OC and apprise patient of potential fetal risks and discuss continuation of pregnanc Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, thoug this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy test **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 study; appeared in 6-12 months' use in another. Bile duct cancer has been reported. **7.** *Carbohydrate and lipid metabolism:* Decrease glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglyceride total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few month and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but increas with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension in user Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pregnanc familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstru cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP may b in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardiovascul disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of migraine development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating caus **10.** *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, ru out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a highe estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenorrhea secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Thos with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, ma occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been associate with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patien with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio of ectop to intrauterine pregnancies is no higher than in nonusers. **12.** *Breast feeding:* OCs may decrease quantity and quality of mil A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after weanin **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable differen in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persis to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precaution** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, wi BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive histor observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor careful in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increas risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC ar troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunctio and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblast anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise patholog of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stoppi for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increas prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increas thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, unaltered fr T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceri phospholipid, reduced serum folate; impaired glucose tolerance, altered plasma trace minerals. Influence of prolonged OC u on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onset climacteric. Cervical chlamydial and gonorrheal prevalence is increased; do not assume OC protects against chlamydial PI HIV seropositivity was associated with OC use. *Information for patients.* See patient labeling. *Drug interactions.* OC effectivene may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramphenic sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, analgesi tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, tricyc antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophe **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebi and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; corona thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; beni adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There evidence of association between the following and OCs, though confirmatory studies have not been done: mesente thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nause vomiting, gastrointestinal symptoms. BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and aft use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargemer secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholesta jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vagir candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted f premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthes nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme nodosum, hemorrhagic eruption, herpes gestationis, hidradenitis suppurativa, hemolytic uremia, malignant hypertensio impaired renal function, acute renal failure, sometimes irreversible, itching, vaginitis, porphyria. PVCs, abnormal EK pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosu rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanom endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been reported aft large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Comple prescribing information should be consulted prior to use and is available on request.**

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Use of urinary luteinizing hormone immunoassays in the assessment of luteal function in infertile women

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It has been suggested that the chronologic date of an endometrial biopsy performed to evaluate luteal adequacy should be based on the date of the luteinizing hormone surge rather than the date of the next menstrual period. Sixty-four infertile women used a urinary luteinizing hormone immunoassay to identify the luteinizing hormone surge; timed serum progesterone level tests and an endometrial biopsy were then performed. An out-of-phase endometrium was identified in 26.6% of cycles dated traditionally and 28.1% of cycles dated from the luteinizing hormone surge. No relationship was identified between progesterone levels and endometrial biopsy results when the next menstrual period was used. When the luteinizing hormone surge was used no progesterone cutoff value could be identified that would reliably distinguish between in-phase and out-of-phase cycles. Use of a urinary luteinizing hormone immunoassay offers no advantage over the next menstrual period and does not lead to better agreement between histologic and chronologic dating. (Am J Obstet Gynecol 1992;166:41-6.)

Key words: Luteal phase defect, progesterone, luteinizing hormone immunoassays, diagnostic test

It is commonly stated that inadequate hormonal preparation of the endometrium for implantation of the blastocyst may be a cause of repetitive spontaneous abortion and infertility.[1-3] This clinical disorder, referred to as luteal phase deficiency, is thought to be caused by a recurrent postovulatory deficiency in the production of progesterone from the corpus luteum.[4] The most widely accepted and clinically applicable method of evaluating corpus luteal function has been a timed luteal phase endometrial biopsy.

Traditionally the chronologic date of the endometrial biopsy has been based on the date of onset of the next menstrual period after the endometrial biopsy and assumes a 14-day luteal phase in all women. It has recently been suggested that midcycle events correlate more accurately with endometrial biopsy results than does the next menstrual period.[5-8] Urinary luteinizing hormone (LH) immunoassays may be useful in evaluating luteal function by allowing a more accurate assignment of the chronologic day of the endometrial biopsy because they use the midcycle LH surge rather than the next menstrual period. This may reduce the number of false positive out-of-phase endometrial biopsy results and possibly avoid further unnecessary evaluation and therapy for luteal phase deficiency. In addition, urinary LH immunoassays may allow more accurate timing of serum progesterone levels in the luteal phase and may provide a more reliable means of identifying the short luteal phase than basal body temperature graphs.

The purposes of this investigation were as follows: (1) to determine whether a urinary LH immunoassay offers any advantage over the next menstrual period in assigning the chronologic date to the endometrial biopsy; (2) to determine whether serial (three) urinary LH-timed serum progesterone levels correlate with results of an endometrial biopsy specimen obtained in the same cycle and whether timed serum progesterone values can be used to discriminate reliably between normal and abnormal cycles as defined by the timed late-luteal phase endometrial biopsy; and (3) to evaluate the relationship between the short luteal phase and LH-timed endometrial biopsy results obtained in the same cycle.

Material and methods

Patient characteristics. Otherwise healthy infertile women seen for evaluation at the Reproductive Endocrine/Infertility Clinic at The University of Medicine and Dentistry of New Jersey–Robert Wood Johnson University Hospital were recruited for this study. Participating subjects had had at least 1 year of unprotected coitus without conception. Each subject had a history of spontaneous, regular ovulatory menses at an interval of 25 to 35 days. Women receiving clomiphene

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citrate, bromocriptine, progesterone vaginal suppositories, or other medications potentially having an effect on the ovarian cycle were excluded. Patients with histories of short luteal phase, luteal phase deficiency, or repetitive spontaneous abortion were also excluded.

Clinical protocol. The study protocol was approved by the Institutional Review Board. After written informed consent was obtained from the subjects, they were asked to use an ovulation prediction kit (QTest, Becton Dickinson, Franklin Lakes, N.J.) each day at home beginning 2 to 3 days before the expected day of ovulation as determined by prior basal body temperature charts or menstrual history. A urine LH test was performed daily (late afternoon or early evening). The day that the urinary LH surge was detected was referred to as LH + 0. Serum progesterone levels were obtained by venipuncture between 7:30 and 10 AM 4 (LH + 4), 7 (LH + 7), and 10 (LH + 10) days after identification of the midcycle urinary LH surge. Occasionally blood was drawn 1 day before or after the recommended day(s) because of scheduling problems. On the same morning as the third progesterone determination (LH + 10) and endometrial biopsy was performed with an endometrial suction curette (Pipelle, Unimar, Inc., Wilton, Conn.) with a tenaculum if needed. Subjects recorded the date of onset of menses (full flow) after the endometrial biopsy.

Tissue obtained from the endometrial biopsy was fixed in 10% buffered formalin, processed in routine fashion, embedded in paraffin, and evaluated by two experienced pathologists in a blinded fashion. Efforts were directed to give as precise an evaluation of postovulatory dating as possible. The two pathologists independently reviewed and dated all slides at the end of the study according to standard criteria and a single-day assignment. The mean of the two histologic cycle days assigned the endometrial biopsy specimen by the two observers was used in the statistical analysis. An out-of-phase biopsy was defined as a ≥3 day lag between endometrial development as determined by the criteria of Noyes and the chronologic date of the biopsy as determined by either the next menstrual period or the LH surge. The date of onset of menses after the endometrial biopsy was assigned as day 28, and the chronologic date of the biopsy was calculated by counting backward. Luteal length was defined as the number of days after the detection of the urinary LH surge up to and including the day before onset of the next menses.[9] Short luteal phase was defined as a luteal phase duration of 11 or fewer days.[10]

Hormone assays. Urinary LH was detected with a widely available ovulation prediction kit (QTest), with all subjects following the instructions provided with the kit. All serum progesterone specimens were centrifuged within 2 hours of collection and stored at −20° C until assayed. Specimens were assayed in the same batch with a commercially available kit (Coat-A-Count, Diagnostics Products Corp., Los Angeles). The intraassay variation for progesterone was 8.3%.

Statistical analysis. Two sample independent Student's t tests were used to compare subsets of subjects. Relationships between continuous variables are summarized by the Pearson product-moment correlation coefficient. To determine whether a linear combination of variables predicts outcome, multiple regression analysis was performed on the data. p Values were considered significant if they were <0.05 two-tailed. All values reported are mean ± SD unless otherwise noted.

Results

Of the 86 subjects who entered the study, 64 completed it by identifying the urinary LH surge and having three serum progesterone level tests and an endometrial biopsy performed in the same cycle. Twenty-two other subjects could not be included in the data analysis for a variety of reasons, including inability to detect an LH surge ($n = 2$), pregnancy during the study cycle ($n = 5$), noncompliance ($n = 3$), endometritis on biopsy ($n = 2$), cervical stenosis ($n = 2$), or incomplete sampling ($n = 8$). Data from five subjects who conceived in the study cycle were excluded from the general data analysis and were analyzed separately.

Mean subject age was 32.1 ± 4.6 years (range 22 to 44 years). The urinary LH surge was detected on mean cycle day 14.75 ± 2.70 (range 10 to 24). The mean luteal days for measurement of serum progesterone were LH + 4.20, LH + 7.11, and LH + 10.04. Mean cycle length was 29.03 ± 3.30 days (range 23 to 38 days), and mean luteal length was 13.27 ± 2.20 days (range 8 to 21 days). Mean serum progesterone values on LH + 4, LH + 7, and LH + 10 were 8.53 ± 3.80, 13.19 ± 6.38, and 11.59 ± 6.50 ng/ml, respectively. The mean sum of the three progesterone levels was 33.41 ± 13.50 ng/ml for all cycles.

The mean histologic day assigned the endometrial biopsy specimen by observer 1 was 23.13 ± 2.74 days and 22.38 ± 2.81 days by observer 2. The mean difference between the two raters was ±0.75 days ($p < 0.05$). The histologic day assigned the endometrial biopsy taken as an average of the two raters was 22.75 ± 2.67 days. Correlation between the observers was high ($r = 0.860$, $p < 0.05$). The observers agreed exactly in 18.8% of specimens and agreed within ±2 days in 89.9% of specimens. The mean chronologic day of the cycle on which the endometrial biopsy occurred was cycle day 23.74 ± 2.10 with the next menstrual period method and cycle day 24.02 ± 0.67 with the urinary LH surge method, or a mean of 0.28 days later with the LH method (not significant). A secretory en-

dometrium was associated with a midluteal (LH + 7) progesterone value >3 ng/ml in 98% of cycles.

When standard criteria (next menstrual period) were used to evaluate each cycle, 26.6% of cycles were out of phase. When the urinary LH surge was used to assign the chronologic day to the endometrial biopsy, 28.1% were out of phase (not significant). There was no difference between mean progesterone values obtained on LH + 4, LH + 7, or LH + 10 in in-phase or out-of-phase cycles as defined by standard criteria (Table I). No significant difference existed between the sum of the three progesterone values obtained in in-phase (34.35 ± 15.23 ng/ml) and out-of-phase (33.04 ± 13.4 ng/ml) cycles as defined by standard criteria.

A significant difference existed between mean progesterone values on LH + 4 when comparing in-phase (9.07 ng/ml) and out-of-phase (6.76 ng/ml) cycles when the urinary LH surge was used to assess the cycle ($p < 0.02$). There was no significant difference between progesterone values on LH + 7 and LH + 10 and the sum of the three progesterone values between in-phase and out-of-phase cycles with the LH surge to evaluate luteal adequacy (Table I).

To explore the possibility that some linear combination of progesterone values may explain the variance in the data, multiple regression-discriminant analysis was used. No distinction between in-phase and out-of-phase cycles based on progesterone determinations obtained on LH + 4, LH + 7, or LH + 10, and the sum of progesterone values for cycles assessed by traditional criteria was identified. When the urinary LH surge was used to assign the chronologic date to the biopsy, only in the case of serum progesterone on LH + 4 was there any correlation ($p < 0.05$) between any linear function of serum progesterone values and luteal adequacy as determined by endometrial biopsy. However, a progesterone cutoff value on LH + 4 with acceptable diagnostic test properties could not be identified. The best serum progesterone cutoff value determined on LH + 4 was 7.0 ng/ml; however, this level showed a sensitivity of 50%, specificity of 79%, positive predictive value of 44%, negative predictive value of 79%, and accuracy of 69%. Using the serum cutoff progesterone value of 7.0 ng/ml as suggested by Daya and Ward[11] on LH + 4 did not allow us to predict in-phase or out-of-phase biopsies with sufficient sensitivity and specificity to permit use of this single luteal progesterone determination to assess luteal function.

A total of five endometrial biopsies were performed during conception cycles. One of these pregnancies spontaneously aborted in the first trimester, a spontaneous abortion rate (20%) similar to that expected in a normal population. Progesterone values on LH + 4, LH + 7, and LH + 10, and the sum of progesterone values were not significantly different in pregnancy versus nonpregnancy cycles. In two conception cycles the endometrial histologic date was >2 days behind the chronologic date of the endometrial biopsy based on the urinary LH surge, suggesting luteal inadequacy.

When luteal phase length was determined with the urinary LH surge, 12 (18.8%) of 64 cycles had short luteal phases (≤11 days) (Table II). Of the 12 women with short luteal phases, 2 (16.7%) of the cycles were out of phase by the LH method and 4 (33%) were out of phase by standard criteria. Of those women with normal luteal length, 30.2% had an out-of-phase endometrial biopsy by LH surge criteria. Mean progesterone values on LH + 4 and LH + 7 did not differ in women with or without short luteal phases. Mean serum progesterone on LH + 10 was significantly lower ($p < 0.05$) for women with short luteal phases (6.46 ± 3.1 ng/ml) when compared with women with luteal length of >11 days (12.37 ± 6.5 ng/ml). Luteal length was positively correlated ($p < 0.001$) with progesterone on LH + 4 and negatively correlated with progesterone on LH + 10 ($p < 0.001$).

Comment

Evaluation of luteal adequacy by endometrial biopsy continues to be a commonly performed investigation in the evaluation of both repetitive spontaneous abortion and infertility. It has been suggested that the chronologic day assigned the endometrium should be correlated to the midcycle LH surge rather than the date of onset of the next menstrual period.[5-8] The conventional approach to determining the chronologic date of the endometrial biopsy by counting backward from the date of the next menstrual period may be flawed because it is clear that only a minority of cycles appear to have a luteal phase length of exactly 14 days,[10] and confusion often exists between postbiopsy spotting and true menses. There may be better agreement between histologic and chronologic dating of endometrial biopsy specimens (and thus a lower rate of false-positive results) when the LH surge is used to assign a chronologic date to the specimen.[8] Urinary LH immunoassays correlate well with serum radioimmunoassays for LH[12] and provide us with a simple, relatively inexpensive, and practical means of identifying the preovulatory LH surge.[13]

Shoupe et al.[7] showed an 84.6% accuracy rate in dating the endometrial biopsy from the serum LH surge and suggested that use of urine LH kits may allow more accurate dating of the endometrial biopsy specimen. Similarly, Li et al.[8] found that use of the prospective method of dating the endometrial biopsy from the LH surge (detected in serum and/or urine) rather than the next menstrual period could reduce the incidence of out-of-phase biopsies from 42% to 10%. These studies suggested that use of the next menstrual period to as-

Table I. Comparison of progesterone values between in-phase and out-of-phase cycles in infertile women

	Next menstrual period method			*LH method*		
	In-phase	*Out-of-phase*	*Significance*	*In-phase*	*Out-of-phase*	*Significance*
No. of cycles (%)	47 (73.4)	17 (26.6)	NS	46 (71.9)	18 (28.1)	NS
Mean progesterone level on LH + 4 (ng/ml)	8.57	8.08	NS	9.07	6.76	$p < 0.02$
Mean progesterone level on LH + 7 (ng/ml)	13.59	14.09	NS	14.40	12.18	NS
Mean progesterone level on LH + 10 (ng/ml)	11.86	10.86	NS	11.35	12.26	NS
Mean progesterone level (ng/ml)	34.35	33.04	NS	34.69	32.02	NS

NS, Not significant.

Table II. Relationship between short luteal phase cycle as determined by QTest and endometrial maturation

Cycle length (days)	*No. of patients*	*Out-of-phase by LH method*		*Out-of-phase by next menstrual period*	
		No.	*%*	*No.*	*%*
≤11	12	2	16.7	4	33.4
>11	52	16	30.8	14	26.9

sign the chronologic date of the endometrial biopsy leads to overestimation of the incidence of out-of-phase cycles and may lead to unnecessary further evaluation and therapy.

Single or multiple serum progesterone level tests continue to be recommended as means of assessing luteal function.[14-19] These tests continue in clinical practice in spite of knowledge that progesterone is secreted from the corpus luteum in an episodic manner such that peripheral serum progesterone values can vary greatly from minute to minute.[20-23] The pulsatility of progesterone release and resultant highly variable serum progesterone concentrations likely explain the difficulty many authors have had in correlating endometrial biopsy findings with serum progesterone levels.[15, 24] These facts led Syrop and Hammond[25] to suggest that the intrinsic variability of progesterone levels in the midluteal phase can be minimized by using timed morning blood for progesterone determinations.

In spite of the potential theoretic advantages that urinary LH immunoassays offer in assessing luteal function, we found no significant difference in the incidence of out-of-phase endometrial biopsies whether traditional criteria (the next menstrual period) or the urinary LH surge was used to assign the chronologic day to the endometrial biopsy. Why daily serum LH determinations by radioimmunoassay[7, 8] and not the identification of the urinary LH surge by QTest decreased false-positive endometrial biopsy results is not readily apparent. Additional variables may have been introduced by allowing subjects to test urine at home and outside the controlled environment of a laboratory. In addition, once daily urine testing may not have permitted accurate identification of the day of the LH surge. It is also possible that the correlation between urinary LH determinations by enzyme immunoassay and serum LH determinations by radioimmunoassay may not be as high as previously reported.[12] The subjectivity and imprecision introduced by dating the endometrium with the standard dating criteria of Noyes may also have contributed to our findings.[26]

We have also attempted to correlate the results of the endometrial biopsy with urinary LH-timed serial morning luteal phase progesterone values in the same cycle. Although serum progesterone levels on LH + 4 did differ between in-phase and out-of-phase cycles when the urinary LH surge was used, no progesterone cutoff value on LH + 4 that could distinguish in-phase and out-of-phase cycles with acceptable sensitivity and specificity could be identified. This finding is consistent with the report of Li et al.,[27] which found that retarded endometrial development was more likely to be associated with a subnormal salivary progesterone profile in the early luteal phase than the late luteal phase. In addition, another recent study found that total pregnanediol excretion was significantly less for luteal phase deficiency cycles during the first 5 days of the luteal phase.[28]

We can only speculate as to why progesterone levels in the early luteal phase and not the midluteal and late luteal phases identified a greater mean progesterone value for in-phase cycles when LH timing was used. That mean progesterone values on LH + 4 did not differ between in-phase and out-of-phase cycles as determined by the next menstrual period may be related to our ability to identify better the early luteal phase with urinary LH assays. Total progesterone production from the corpus luteum as determined by daily serum or salivary progesterone assays is lower in cycles with an out-of-phase endometrial biopsy.[29] It has been demonstrated that progesterone secretion in the early luteal phase is stable and does not demonstrate rapid fluctuations over 24 hours as in the midluteal and late luteal phases.[20, 21] It may be that decreased pulsatility of progesterone secretion in the early luteal phase permitted us to detect the higher levels of progesterone in in-phase cycles more readily. It is possible that the higher serum progesterone levels in the early luteal phase led to a direct improved effect on endometrial development and maturation.

The role that the short luteal phase plays in infertility, if any, is uncertain. It is not known whether a short luteal phase cycle is by necessity abnormal in terms of luteal function. Short luteal phases are not uncommon and may represent a variant in the spectrum of normal luteal function.[10] Short luteal phases occur with the same frequency in normal and infertile populations.[9, 10] Our finding of an in-phase endometrial biopsy (by both criteria) in the majority of patients with a short luteal phase supports the concept that identifying a cycle with a short luteal phase does not necessarily signify that luteal function, as determined by endometrial biopsy, is adversely affected.

We have demonstrated in this study that, with the exception of serum progesterone on LH + 4, both single and serial luteal progesterone levels timed by urinary LH immunoassay correlate poorly with the assessment of luteal function as determined by the endometrial biopsy. Our data suggest that the practice of using luteal phase serum progesterone values and even well-timed serial morning specimens to evaluate luteal adequacy provides little meaningful information and correlates poorly with histologic findings on endometrial biopsy. In addition, identification of the urinary LH surge by QTest did not result in better agreement between chronologic and histologic dating of the endometrial biopsy and did not appear to offer any advantages over traditional dating techniques.

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Intracerebral, aortic, and umbilical artery flow velocity waveforms in the late-first-trimester fetus

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OBJECTIVES: Our objectives were to determine the success rate in obtaining flow velocity waveforms in the first-trimester fetal circulation and to establish possible preferential flow to the fetal cerebrum at this early stage of gestation.

STUDY DESIGN: Flow velocity waveform recordings were made in the umbilical artery, fetal descending aorta, and fetal intracerebral arteries in 30 normal pregnancies between 11 and 13 weeks of gestation.

RESULTS: Technically acceptable waveforms were obtained from the descending aorta in 15 fetuses, from the intracerebral circulation in 17 fetuses, and from the umbilical artery in all 30 fetuses. Absent end-diastolic velocities in the descending aorta and umbilical artery were associated with forward flow throughout the cardiac cycle in intracerebral arteries.

CONCLUSION: A relatively low cerebral vascular resistance in the late-first-trimester normal fetus is suggested. (Am J Obstet Gynecol 1992;166:46-9.)

Key words: Transvaginal ultrasonography, pulsed Doppler, fetal circulation

Third-trimester pregnancies are characterized by forward end-diastolic flow velocities in umbilical and fetal arterial vessels that reflect a low-resistance feto-placental unit.[1] After the introduction of a Doppler method for recording flow velocity waveforms in the human fetal internal carotid artery in third-trimester pregnancies,[2] evidence was provided of reduced cerebral vascular resistance in cases of intrauterine growth retardation that is due to placental insufficiency.[2-4]

Transvaginal ultrasonography allows more detailed visualization of fetal structural development in early pregnancy[5] and as a result is a potential tool for the Doppler recording of blood flow in first-trimester fetal vessels such as the common and internal carotid artery, the descending aorta, the cardiac outflow tract, and the umbilical artery. A preliminary study on umbilical artery flow velocity waveforms with transabdominal continuous-wave Doppler ultrasonography has demonstrated high pulsatility as a result of absent end-diastolic flow velocities that reflect high umbilical-placental vascular resistance.[6] Because early pregnancy is particularly characterized by rapid growth of the fetal

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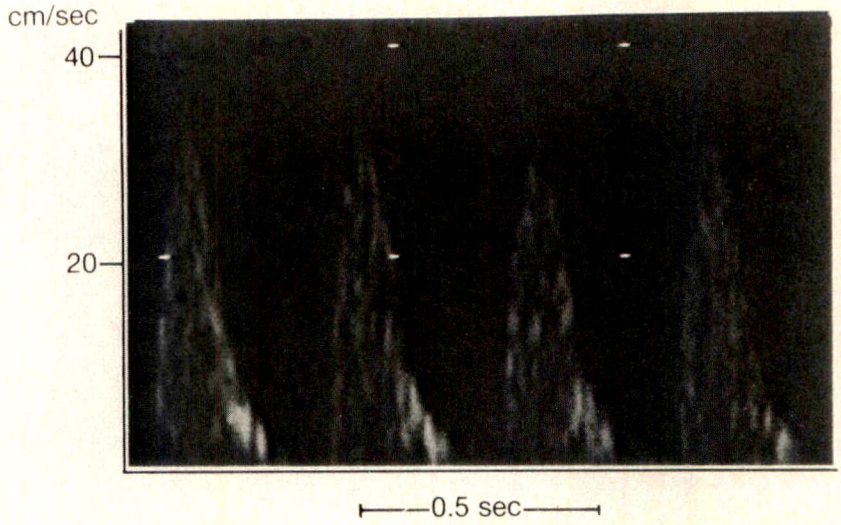


Fig. 1. Flow velocity waveforms from descending aorta in the late-first-trimester normal fetus.

head, it is unknown whether a relatively low vascular resistance is present at the cerebral level.

The objective of this study was twofold: (1) to determine the success rate in obtaining technically acceptable flow velocity waveforms in the fetal intracerebral circulation, the descending aorta, and the umbilical artery in late-first-trimester pregnancies and (2) to relate intracerebral artery flow velocity waveforms to those obtained from the descending aorta and the umbilical artery to establish possible preferential blood flow to the fetal cerebrum at this early stage of gestation.

Material and methods

Thirty normal singleton pregnancies were included in the study. Maternal age varied between 23 and 38 years (median 27 years). Gestational age ranged between 11 and 13 weeks (median 12 weeks). All pregnant women consented to participate in the study. The study protocol was approved by the hospital's ethics committee. A combined transvaginal real-time and pulsed Doppler system (Hitachi E.U.B. 450, Medical Cooperation, Tokyo) with a carrier frequency of 3.5 and 6.5 MHz was used. The system operates at power outputs of <100 mW/cm^2 spatial peak–temporal average in both imaging and Doppler modes by the manufacturer's specifications. Each woman was included in the study once. Doppler studies were performed by one examiner (J.W.W.). Maximum flow velocity waveforms were collected from the fetal intracerebral circulation, the descending aorta, and the umbilical artery. Intracerebral artery flow velocity waveforms were documented on a transverse cross section through the lower part of the fetal cerebrum; this showed a heart-shaped cross section of the brain stem with the anterior lobes representing the cerebral peduncles.[2] No distinction could be made between internal carotid, middle, or anterior cerebral artery waveforms. Flow velocity waveforms from the lower thoracic part of the fetal descending aorta were recorded from a sagittal cross section through the fetal trunk that displayed a major section of the fetal spine.[7] The flow velocity waveform in the umbilical artery was obtained from a free-floating loop of the umbilical cord.[8] Sample volume length was 0.1 to 0.3 cm. The pulsatility of the waveform was expressed by the pulsatility index, which is derived by dividing the difference between peak systolic and end-diastolic velocity by the mean flow velocity over the entire cardiac cycle.[9] Heart rate (beats per minute) was calculated from peak velocity time intervals in the descending aorta. All Doppler studies were performed with the woman in the semirecumbent position and during fetal apnea, because fetal breathing movements modulate blood flow velocity waveforms.[10] The total examination time was limited to 10 minutes in each instance.

All flow velocity waveforms were recorded on hard copies. A microcomputer (Olivetti M 24, Olivetti BV, Leiden) linked to a graphics tablet was used for analysis of Doppler recordings. An average of three consecutive flow velocity waveforms of similar appearance and with the highest velocity was used to establish each value. Data are presented as mean ± 1 SD.

Results

Technically acceptable flow velocity waveforms were obtained from intracerebral arteries in 21 fetuses (70%), from the descending aorta in 15 (50%), and from the umbilical artery in all of the 30 fetuses studied. Although flow velocity waveforms in the descending aorta (Fig. 1) and umbilical artery (Fig. 2) display ab-

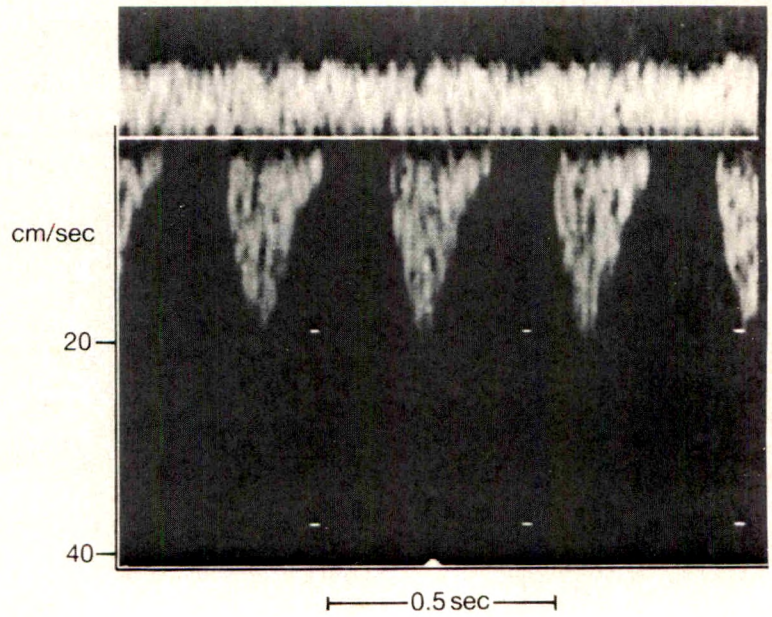


Fig. 2. Flow velocity waveform from umbilical artery in late-first-trimester normal fetus.

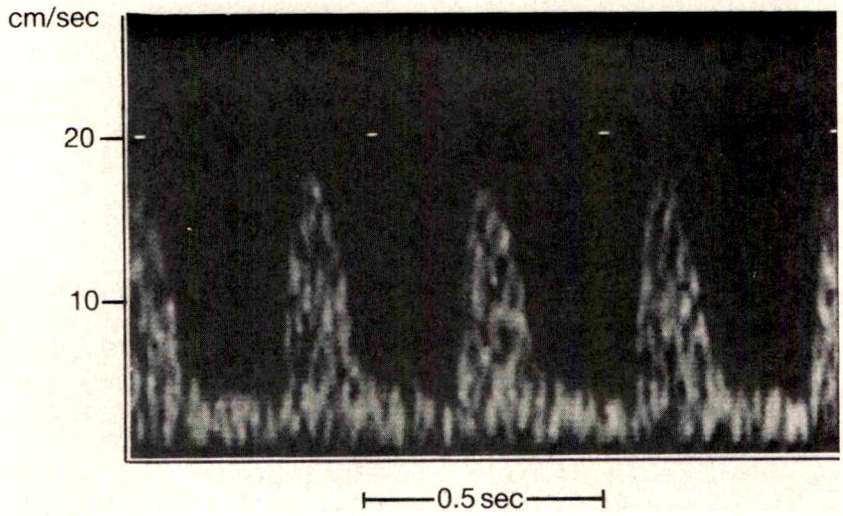


Fig. 3. Flow velocity waveform from intracerebral arterial circulation in late-first-trimester normal fetus.

sent end-diastolic flow, flow velocity waveforms in the intracerebral arteries are characterized by forward flow throughout the cardiac cycle (Fig. 3). The pulsatility index was 2.5 ± 0.2 in the fetal descending aorta, 2.6 ± 0.3 in the umbilical artery, and 2.0 ± 0.2 in the intracerebral artery. Fetal heart rate ranged between 146 and 188 beats/min with a mean value of 170 ± 6 beats/min. No statistically significant correlation existed between flow velocity in all three vessels and the heart rate.

Comment

The objective of this study was to establish the nature of intracerebral, aortic, and umbilical flow velocity waveforms in late-first-trimester pregnancies. Flow velocity waveform recording was successful in more than half of the women examined. Fetal motor activity, particularly of the trunk, was the main cause of failure to obtain acceptable waveforms. Exact identification of the intracerebral vessels under investigation was not possible, although positioning of the sample volume anterior to the cerebral peduncles should limit the origin of the waveforms to the internal carotid and the middle and anterior cerebral arteries. Intracerebral forward end-diastolic flow, as opposed to absence of end-diastolic flow in the descending aorta and umbilical artery, reflects comparatively lower vascular resistances in the fetal cerebrum. This is supported by the observation

that the mean intracerebral pulsatility index value in this study is only marginally higher than that established in late-second-trimester pregnancies.[11] In contrast, a similar comparison for the mean pulsatility index in the descending aorta and umbilical artery revealed that late-first-trimester pregnancies are associated with considerably higher values, i.e., by factor 1.4 for the descending aorta and by factor 2.0 for the umbilical artery.

Our late-first-trimester fetal heart rates are higher than those observed in second- and third-trimester pregnancies; this is due to parasympathetic immaturity.[12] Heart rate dependency was established in normal third-trimester pregnancies for all three fetal vessels.[13] The rather narrow heart rate distribution may explain the absence of heart rate dependency of fetal flow velocity waveforms in this study.

It can be concluded that the transvaginal Doppler technique can be used to study the early human fetal circulation. The intracerebral velocity waveforms suggest a relatively low vascular resistance at fetal cerebral level in early gestation.

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Editors' note

The American Journal of Obstetrics and Gynecology introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the Journal should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case report, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Ovarian metastases in stage IB carcinoma of the cervix: A Gynecologic Oncology Group study

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Surgical and pathologic findings at laparotomy for radical hysterectomy in 990 patients with clinical stage IB carcinoma of the cervix were analyzed to determine the frequency of metastases to the ovary. Ovarian spread was identified in 4 of 770 (0.5%) patients with squamous carcinoma and 2 of 121 (1.7%) with adenocarcinoma. No patients with adenosquamous carcinoma (n = 82) or other histologic types (n = 17) had ovarian metastases. Although the frequency of metastases was greater among patients with adenocarcinoma, this was not statistically significant (p = 0.19, Fisher's exact test). All 6 patients with ovarian metastases had other evidence of extracervical disease. Three underwent radical hysterectomy and bilateral salpingo-oophorectomy. Of these, one patient received extended field radiotherapy and died of disease 18 months after diagnosis. Two patients, one treated with combination chemotherapy and one with no adjunctive therapy, are alive without evidence of disease at 59 and 62 months, respectively. Three patients underwent exploratory laparotomy with salpingo-oophorectomy and lymphadenectomy without hysterectomy. All three patients died of disease at 2, 3, and 30 months; the first and last patient received adjunctive radiotherapy. Not all patients underwent oophorectomy. Of 347 patients with at least unilateral ovarian preservation, no postoperative pelvic radiotherapy, and no gross extracervical disease or metastasis to the paraaortic nodes, pelvic recurrence developed in 16. There was no excess of pelvic recurrences in patients with adenocarcinoma (0/41) or adenosquamous carcinoma (1/29, 3.4%) when compared with those with squamous carcinoma (15/270, 5.6%). This suggests no excess of occult ovarian metastases in nonsquamous tumors of the cervix. There is no evidence in these data of an increased risk of ovarian preservation in patients with stage IB carcinoma of the cervix with no gross extracervical disease. (AM J OBSTET GYNECOL 1992;166:50-3.)

Key words: Ovarian metastases, carcinoma of the cervix

Early stage squamous carcinoma of the cervix rarely metastasizes to the ovary. Preservation of the ovaries in premenopausal women undergoing surgical therapy for early invasive cervical cancer has been one of the foundations of practice in gynecologic oncology since McCall et al.[1] published the results of their 9-year retrospective study in 1958.

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Mann et al.[2] recently reported two cases of ovarian metastases in stage IB adenocarcinoma of the cervix. They cited evidence[3] suggesting that the practice of ovarian preservation during surgery for early adenocarcinoma of the cervix was not as safe as had been established in squamous lesions. The purpose of the current study was to determine whether there is an increased risk of ovarian metastases in clinical stage IB adenocarcinoma and adenosquamous carcinoma of the cervix when compared with squamous carcinoma.

Material and methods

Women with previously untreated histologically confirmed International Federation of Gynecology and Obstetrics stage IB cervical cancer were eligible for a prospective surgical-pathologic study conducted by the Gynecologic Oncology Group.[4] Eligibility requirements included a signed informed consent and surgical procedure as noted here. Patients were ineligible if they had received chemotherapy or radiotherapy, had other noncutaneous malignancies (melanoma excepted), had greater than International Federation of Gynecology

and Obstetrics stage IB lesions, or were poor operative risks. At surgery, patients underwent a thorough examination while they were under anesthesia, peritoneal fluid was obtained for cytologic evaluation on entering the abdomen, and radical hysterectomy and bilateral pelvic and paraaortic lymphadenectomy as described by Meigs[5] were performed. For patients with evidence of tumor confirmed by frozen section in tissues other than the uterus, cervix, or pelvic lymph nodes, only exploration and appropriate biopsies were required. In most of these cases radical surgery was not completed and radiotherapy was given. Similarly, patients with fixed, histologically positive, nonresectable pelvic nodes were required to have only paraaortic lymph node biopsy and exploration of the abdomen and pelvis before definitive radiotherapy. Removal or nonremoval of the ovaries was determined by the primary surgeon. There was no prospective attempt to establish guidelines for the conservation or removal of ovarian tissue. Although menopausal status was not coded in the primary study, 209 patients (21%) were >52 years of age.

Representative slides of the primary lesion and additional positive sites were reviewed by the Gynecologic Oncology Group Pathology Committee and note was made of the depth of invasion and the presence or absence of capillary space involvement.

For our study, data from Gynecologic Oncology Group statistical files were reviewed for those cases in which spread to the ovaries at the primary operation was identified. Fisher's exact test[6] was used to measure the association between cell type and ovarian metastasis.

Results

Between May 1981 and April 1984, 1125 patients were entered into this surgical-pathologic study. Of these, 121 were excluded preoperatively for reasons including wrong stage, minimal tumor invasion, inadequate pathologic material to confirm eligibility, and late registration. There were 14 postoperative exclusions; 12 had incorrect or double primaries and the remaining two were excluded because their institution failed to provide the necessary documentation. The remaining 990 patients were evaluable for this study and include 49 patients who underwent exploration but did not have all the required surgical procedures performed.

Of the evaluable patients, 770 (77.9%) had squamous lesions, 121 (12.2%) had adenocarcinoma, 82 (8.2%) had adenosquamous carcinoma, and 17 (1.7%) had other histologic types of tumor.

One hundred eleven patients (11.2%) did not undergo radical hysterectomy because of the finding at laparotomy of gross extracervical disease or metastases to the paraaortic nodes. Eighty-nine of these patients had squamous cancer and 22 had nonsquamous lesions. There were 94 (84.7%) patients who received definitive radiotherapy. Four of these patients (3.6%) had ovarian and other metastases at the time of laparotomy. Three had squamous carcinoma and one had adenocarcinoma. Details of these four cases are found in Table I. All had extensive extracervical spread and all but one received postoperative radiotherapy. All four patients died of cancer at intervals of 2, 3, 18, and 30 months after exploratory surgery.

A total of 833 patients with no gross extracervical disease or metastasis to the paraaortic nodes underwent radical hysterectomy and lymphadenectomy. Two of these patients were found to have ovarian metastases at laparotomy (0.2%), one with squamous carcinoma and one with adenocarcinoma. These metastases were 3 and 6 cm in diameter and were unilateral in both cases. One patient with squamous carcinoma did not receive adjunctive radiotherapy and was alive without disease 62 months after surgery. The patient with adenocarcinoma received chemotherapy with cisplatin, doxorubicin, and cyclophosphamide and was alive and well 59 months postoperatively.

Of the 833 patients who underwent radical hysterectomy, 439 (52.7%) also had bilateral salpingo-oophorectomy. Unilateral adnexectomy with preservation of the contralateral ovary was performed in 91 patients, and three had a solitary residual ovary removed at the time of radical hysterectomy. Thus 391 patients undergoing radical hysterectomy had at least one gonad preserved. Of this group 47 received postoperative pelvic radiotherapy, leaving 344 patients at theoretic risk for recurrence from undetected micrometastases in the residual ovary. In 16 of these 344 patients (4.7%) recurrence with a pelvic component did develop. However, there was no excess of pelvic recurrence in patients with adenocarcinoma (0/41) or adenosquamous carcinoma (1/29, 3.4%) when compared with those with squamous carcinoma (15/267, 5.6%). A few patients in this series underwent ovarian transposition and would therefore be at risk for abdominal recurrence if ovarian metastases were present. However, there was no observed difference in the rate of abdominal recurrence by histologic type.

Overall, ovarian metastases were observed in 6 of 990 patients in this study, for a prevalence rate of 0.6%. The distribution of ovarian metastases varied according to the histologic type of tumor. Among 770 patients with squamous cell carcinomaa, four, or 0.5%, had ovarian spread. This contrasts with two of 121 (1.7%) patients with adenocarcinoma and none of 99 with adenosquamous carcinoma or other histologic types. Although metastases are more common in adenocarcinoma and adenosquamous carcinoma in this series, no statistical significance was associated with this difference ($p = 0.19$, Fisher's exact test).

Characteristics of the six patients with clinical stage

Table I. Ovarian metastases in stage IB carcinoma of the cervix

Age (yr)	*Histologic type*	*Surgery*	*Recurrence (mo)*	*Status*	*Survival*	*Other metastases*
35	Squamous	Lap, RT	3.0	DOD	44.9	Pelvic and paraaortic nodes, peritoneal wash
44	Squamous	Lap, RT	3.1	DOD	4.2	Pelvic and paraaortic nodes, fallopian tube
62	Squamous	Radical RT	18.0	DOD	30.1	Pelvic and paraaortic nodes peritoneal wash
24	Adenocarcinoma	Lap, RT	2.2	DOD	2.2	Fallopian tube, appendix, parametrium
44	Squamous	Radical	61.8*	NED	61.8*	Parametrium
34	Adenocarcinoma	Radical, Chemorx	58.6*	NED	58.6*	Fallopian tube

Lap, Laparotomy and bilateral oophorectomy only; *RT*, postoperative radiotherapy; *DOD*, dead of disease; *Radical*, radical hysterectomy, bilateral salpingo-oophorectomy, and lymphadenectomy; *Chemorx*, combination chemotherapy; *NED*, alive with no disease.

*Patient is alive with no evidence of disease as of time indicated.

IB carcinoma of the cervix and ovarian metastases are outlined in Table I.

Nonmetastatic ovarian neoplasms identified in this group of patients included one patient with a Sertoli cell tumor and contralateral serous cystadenoma, four patients with papillary cystadenofibromas, and one each with a Brenner tumor, adrenal rest, and mucinous cystadenoma of low malignant potential. These patients represented 0.9% of the total in this series.

Comment

International Federation of Gynecology and Obstetrics stage IB carcinoma of the cervix may be treated by radical hysterectomy or pelvic radiotherapy with similar rates of disease control.[7] In young women radical hysterectomy is often the preferred method of therapy because normal vaginal function can be maintained. The salvage of ovarian function is a second advantage of surgical therapy. McCall et al.[1] demonstrated that the risk of ovarian involvement in early squamous lesions of the cervix is low and that gonadectomy is not required in the absence of visible metastases. This principle of ovarian preservation has been applied in the case of early adenocarcinoma of the cervix by many clinicians. Mann et al.[2] challenged this practice, suggesting that its safety had not been established in nonsquamous lesions of the cervix.

The true incidence of ovarian metastases in stage IB adenocarcinoma of the cervix is unknown and would require microscopic examination of all ovarian tissues at the time of radical hysterectomy. However, some information is available regarding the finding of visible metastases. Kjorstad and Bond[8] described two patients in a series of 150 (1.3%) with ovarian spread in the absence of lymph node metastases. Both underwent oophorectomy, radical hysterectomy, and postoperative pelvic radiotherapy and are free of recurrence. Tabata et al.[3] reported two cases (7.7%) of ovarian metastases among 26 patients undergoing radical hysterectomy and pelvic lymphadenectomy for stage IB adenocarcinoma of the cervix. This incidence was greater than that for squamous lesions (none in 122 cases). Survival information was not presented. In a series of autopsies[3] on patients with advanced disease who had received radiotherapy, chemotherapy, or no treatment, there was a significantly greater finding of ovarian spread among subjects with adenocarcinoma (22 of 77, 28.6%) when compared with those with squamous tumors (104 of 597, 17.4%, $p < 0.02$).

Mann et al.[2] cited two cases of stage IB adenocarcinoma of the cervix with ovarian metastases. The first patient underwent bilateral oophorectomy for the incidental finding of endometriosis and was found to have microscopic metastases in both ovaries without other extracervical disease. She received postoperative radiotherapy and was free of disease at 36 months. The second patient had bilateral pelvic node involvement and microscopic extension into the anterior vaginal fornix. She was alive and well 42 months after surgery and radiotherapy. On the basis of these two cases and a review of the literature, these authors ". . . adopted a policy of recommending oophorectomy at the time of radical hysterectomy and pelvic node dissection for adenocarcinoma of the cervix."[2]

Kaminsky and Norris[9] identified neoplasms in ovaries of 25 of 161 women with endocervical adenocarcinoma either at the time of or subsequent to surgery for this condition. Ten patients had metastases from the cervical primary and, although stage was not discussed, there was generalized pelvic and peritoneal involvement in seven of these cases.

In this report the incidence of ovarian metastases among subjects with squamous carcinomas of the cervix was 0.5%. This finding is nearly identical to that of Baltzer et al.[10] (4/749, 0.5%) and Shingleton and Orr[11] (1/258, 0.4%) and confirms the safety of ovarian pres-

ervation in squamous cancers. All patients with ovarian metastases had evidence of other extracervical disease. Although ovarian metastases were observed more frequently among patients with adenocarcinomas, the absolute incidence of ovarian spread remained very low. Additionally, those patients with isolated ovarian metastases did well when radical hysterectomy was completed in this series of patients. There is no conclusive evidence from these data suggesting an increased risk associated with ovarian preservation in patients who have no gross extracervical disease.

No increased rate of pelvic recurrence could be demonstrated in this series of nonovariectomized patients with adenocarcinoma when compared with those with squamous tumors. Therefore it would seem reasonable to conserve normal-appearing ovaries in young women undergoing radical hysterectomy for treatment of cervical adenocarcinoma and adenosquamous carcinoma, providing that no other extracervical spread is present and that the patient understands the small risk of undetected ovarian spread.

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Describing and interpreting 24-hour blood pressure patterns in physiologic pregnancy

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The time course of blood pressure in clinically healthy (pregnant and nonpregnant) women was followed by automatic ambulatory monitoring. Chronobiologic methods revealed the time course of dynamic rhythm characteristics as a function of gestational age. Differences were found between nonpregnant and pregnant women with an overall lowering during pregnancy of the rhythm-adjusted midline estimating statistic of rhythm (mesor). (Am J Obstet Gynecol 1992;166:54-60.)

Key words: Blood pressure, chronobiology, hypertension, pregnancy

The physiologic pattern of blood pressure consists of 80,000 to 140,000 tensions that take place beat to beat during the day-night cycle. Thus, so-called casual measurements[1] should be replaced by noninvasive automated monitoring to improve clinical assessment of the constitutive sequence of the 24-hour blood pressure pattern. However, the automated monitoring has to be biometrically interpreted, considering that blood pressure is a rhythmic variable showing a circadian-dependent periodicity.[2-8] Interpretation criteria are particularly crucial for pregnancy because blood pressure shows changes peculiar to each stage of gestation.[9-16] On the other hand, the designation of normotensive or hypertensive pregnancies may be important for mothers and babies, whose 24-hour blood pressure pattern shows a chronobiologic separation according to the positive or negative family history.[17]

Our study was designed to approach the 24-hour blood pressure pattern in physiologic pregnancy by describing and interpreting time-qualified variability with particular reference to the periodic pattern.

Material and methods

Subjects and protocol. Thirty pregnant women and thirty nonpregnant women were investigated (Table I). These subjects were stratified from a large sample of randomly investigated individuals. Their participation was allowed because they were classified *a posteriori* to be clinically healthy subjects in accordance with extensive clinical checkups. Furthermore, the pregnant women were all primiparous women not at risk of hypertension and with no history of familial and personal high blood pressure. The pregnant women had uneventful gestations and were vaginally delivered of term infants with Apgar scores >7.

Table I. Clinical data in nonpregnant and pregnant women

Characteristics	*Nonpregnant women*	*Pregnant women*
No. observations	30	30
Age (yr)	20-25	20-26
Height (cm)	176 ± 4	166 ± 5
Weight (kg)	58 ± 4	57 ± 5*
Casual blood pressure		
Systolic (mm Hg)	110-170	105-115
Diastolic (mm Hg)	70-80	65-75

Blood pressure values given as range.
*Before pregnancy.

The 24-hour blood pressure monitoring in pregnant women was repeated in each trimester of pregnancy at 8 to 10, 18 to 20, and 32 to 34 gestational weeks, respectively. Both the nonpregnant women and pregnant women were hospitalized to be synchronized to light-dark alternation (lights on 7 AM; lights off 11 PM) and meal-timing schedule (breakfast 8:30 AM; lunch noon; dinner 6:30 PM) by using a noninvasive ambulatory automated recorder (Space Labs, ICR, Redmont, Washington) that took measurements (oscillometric technique) for systolic and diastolic blood pressure at programmable intervals.

Biometric analysis. Individual time data series were analyzed with methods of noninferential and inferential biometry.

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Table II. Reference limits for cumulative values of 24-hour systolic and diastolic blood pressure pattern in nonpregnant and pregnant women

Groups	SBP (mm Hg)		DBP (mm Hg)	
	Mean	90% TL	Mean	90% TL
Nonpregnant women	118	93; 143	69	50; 88
Pregnant women				
First trimester	102*	87; 117	64*	44; 84
Second trimester	105*	81; 129	64*	44; 84
Third trimester	109*	85; 133	66*	49; 83

TL, Tolerance limits; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure.
*$p < 0.001$ by t test in contrast to nonpregnant women.

Table III. Summary of outcomes by frequency distribution analysis for 24-hour systolic and diastolic blood pressure pattern in nonpregnant and pregnant women

Parameters	Nonpregnant women		Pregnant women by trimester					
			First		Second		Third	
	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
Median (mm Hg)	110	70	101*	61*	112*	70†	125‡	70*
Mode (mm Hg)	120	70	98	57	105	60	105	70
Lower value (mm Hg)	70	35	57	38	70	40	70	40
Higher value (mm Hg)	172	105	177	120	150	95	175	95
Range (mm Hg)	95	70	120	82	80	55	105	55
Variation coefficient (%)	12	16	14	19	12	18	13	15
Q1 Lower quartile (mm Hg)	110	62	91	57	97	56	100	59
Q3 Upper quartile (mm Hg)	130	79	110	71	111	72	118	74
Quartile range (mm Hg)	20	17	19	14	14	16	18	15
97th percentile (mm Hg)	148	90	131	90	131	87	139	83
Classes (5 mm Hg interval)								
Number	19	14	21	16	16	11	21	11
Lower class (mm Hg)	70	35	55	35	70	40	70	40
Upper class (mm Hg)	165	105	175	120	150	95	175	95
Class range (mm Hg)	95	70	120	85	80	55	105	55

SBP, Systolic blood pressure; *DBP*, diastolic blood pressure.
*$p < 0.001$ by median test in contrast to nonpregnant women.
†$p < 0.01$.
‡$p = 0.005$.

Noninferential biometry refers to the analysis of the distribution for blood pressure values estimated independently of their temporal qualification (macroscopic cumulative biometry) or qualified by their daily temporality (macroscopic temporal biometry). Inferential biometry (microscopic temporal biometry) refers to the analysis of the circadian rhythmicity with the Single Cosinor method[18] and the Single Cosint (combination of cosine and integration) procedure.[19]

The single cosinor method is a periodic regression analysis fitting a 24-hour cosine curve to raw data and defining the optimal waveform by means of the least squares method. The cosinor procedure uses this equation:

$$Yt = M + A \cdot \cos(\omega \cdot t + \phi) \qquad (1)$$

where M (mesor, acronym of midline estimating statistic of rhythm) is the rhythm-adjusted mean; A (am-

Table IV. Time-qualified measures of central location (mean) and dispersion (SD) and 90% tolerance intervals for 24-hour systolic and diastolic blood pressure pattern in nonpregnant and pregnant women

	Nonpregnant women						*Pregnant women by trimester*					
	SBP			*DBP*			*First*					
							SBP			*DBP*		
Time (clock hr)	*Mean*	*SD*	*90% TI*	*Mean*	*SD*	*90% TI*	*Mean*	*SD*	*90% TI*	*Mean*	*SD*	*90% TI*
01	112	12	20	64	12	19	89	7	14	63	14	27
02	111	14	23	68	11	19	93	10	21	58	6	11
03	113	13	23	69	11	18	91	14	27	60	8	16
04	112	14	24	68	10	17	93	8	15	52	6	10
05	114	14	24	70	11	18	87	5	10	53	6	12
06	115	14	24	71	11	18	91	14	26	52	8	15
07	117	15	26	73	10	17	96	11	20	57	7	14
08	119	15	26	72	10	17	103	8	14	66	11	22
09	122	18	30	72	10	16	99	12	24	68	12	25
10	120	16	27	73	13	22	103	9	17	69	7	13
11	122	16	27	74	11	18	106	11	21	73	12	23
12	123	17	28	75	11	18	110	10	19	70	16	30
13	121	13	21	72	10	17	105	10	18	65	12	22
14	120	12	21	69	10	16	103	13	24	62	11	20
15	120	15	26	72	11	18	102	19	34	63	8	14
16	123	14	24	74	11	19	102	17	32	66	14	26
17	125	15	25	74	10	16	104	13	24	67	14	26
18	122	13	22	73	11	18	105	6	11	64	8	16
19	122	15	25	71	11	19	107	15	28	65	10	17
20	120	13	21	70	10	17	108	10	19	63	13	23
21	120	16	21	70	10	18	102	9	16	62	10	18
22	118	14	23	71	12	20	101	14	25	67	17	31
23	116	12	21	70	11	15	103	14	26	61	9	18
24	115	13	21	68	11	19	96	8	16	54	10	20

SBP, Systolic blood pressure; *DBP*, diastolic blood pressure; *TI*, tolerance interval.

plitude) represents the extent of oscillatory wave from mesor; ϕ (acrophase) is the timing of the wave with reference to local midnight; t is a fraction of the time period (τ); ω is the angular frequency given by $2\pi/\tau$. The dispersion limits for mesor, amplitude, and acrophase are computed with the population-mean cosinor.[20] The confidence limits are estimated as SEM for mesor and 95% confidence limits for amplitude and acrophase. The Single Cosint method is an integral periodic analysis that estimates the parameter aesor (acronym of area estimating statistic of rhythm), that is, the surface covered by the sinusoidal wave of a given biologic rhythm. The cosint method uses this formula:

$$\text{Aesor} = \int_{t0}^{t24} [M + A \cdot \cos(\omega \cdot t + \phi)]\, dt \qquad (2)$$

where the coefficients have the same meaning as in equation 1. The overall aesor estimates the baric impact as the total number of millimeters of mercury that relate to the systolic and diastolic blood pressure circadian cycle.[21] The aesor may be estimated in a fraction of the circadian time such as diurnal and nocturnal hours (partial aesor). The diurnal and nocturnal aesors differ in relation to the acrophase location. The dispersion limits for the aesor were estimated as SEM.

The sample-related estimates in both the noninferential and inferential procedures were further analyzed to derive the reference limits as 90% tolerance limits (90% TL), herein intended as the reference intervals that include 90% of distribution with 90% of probability. The 90% TL were computed with this formula:

$$90\%\ \text{TL} = M + SD \cdot t_{(0.1)\,(df)} \qquad (3)$$

where M and SD are respectively the mean and standard deviation of the sample distribution, and t is the integral of Student's distribution for a probability $(1 - \alpha)$ of 0.1. *df* is the symbol of the degrees of freedom given by $n - 1$ observations.[22-24]

Comparative statistics. The statistical differences between nonpregnant women and pregnant women were analyzed by different procedures such as the Student *t* test, median test, discriminant analysis, and parameter test, respectively, for the equality of the means, medians, gaussian distribution, and rhythmometric parameters.

Results

Macroscopic cumulative biometry. The analysis of cumulative means and their reference limits for both the 24-hour systolic and diastolic blood pressure patterns is displayed in Table II. The biometric estimates document that the overall mean for systolic and dia-

Pregnant women by trimester												
Second						*Third*					
SBP			*DBP*			*SBP*			*DBP*		
Mean	*SD*	*90% TI*	*Mean*	*SD*	*90% TI*	*Mean*	*SD*	*90% TI*	*Mean*	*SD*	*90% TI*
98	17	33	56	15	30	103	14	25	62	6	10
101	11	20	58	9	15	104	16	28	62	11	19
100	12	23	57	7	14	100	12	21	64	14	25
99	16	32	62	16	31	111	44	78	65	28	50
100	6	12	56	6	12	109	13	24	64	8	15
95	16	31	61	17	37	98	11	21	58	9	15
98	15	27	58	11	21	98	13	22	61	12	21
104	14	26	66	14	28	104	15	27	66	12	21
106	13	23	63	11	21	109	12	21	67	7	12
109	9	17	66	7	13	113	8	15	72	10	17
113	12	23	72	10	18	124	26	46	73	14	24
114	11	21	77	13	24	113	10	18	71	9	15
119	19	34	76	13	23	116	14	25	70	6	11
118	11	20	72	11	20	115	13	23	68	8	14
116	14	24	69	14	25	112	4	27	68	8	15
117	14	27	73	14	25	108	11	20	66	8	14
11	15	27	70	13	23	108	12	21	71	11	18
122	18	33	74	10	19	109	15	26	70	10	17
115	12	22	78	10	17	110	19	34	68	12	20
118	15	27	72	11	20	111	10	18	71	6	11
112	16	28	71	9	17	111	13	24	71	12	21
108	14	20	70	14	25	106	11	19	70	5	9
103	11	19	66	13	23	107	10	18	66	8	14
97	6	12	62	13	25	99	13	23	60	10	18

Table V. Intertime variability for time-qualified mean levels of 24-hour systolic and diastolic blood pressure pattern in nonpregnant and pregnant women

	Nonpregnant women		*Pregnant women by trimester*					
			First		*Second*		*Third*	
Parameters	*SBP*	*DBP*	*SBP*	*DBP*	*SBP*	*DBP*	*SBP*	*DBP*
24-hour mean (mm Hg)	119	70	101	63	108	67	109	66
SD (mm Hg)	4	3	6	6	8	7	6	5
Peak level (mm Hg)	126	75	114	75	122	78	124	73
Trough level (mm Hg)	111	64	87	49	90	46	98	54
Range (mm Hg)	15	11	27	26	32	32	26	19
Variation coefficient (%)	3	4	6	10	8	11	5	7

First trimester, 8 to 10 wk; Second trimester, 18 to 20 wk; Third trimester, 32 to 34 wk; values rounded to nearest unit. *SBP*, Systolic blood pressure; *DBP*, diastolic blood pressure.

stolic blood pressure is reduced in pregnant women as compared with nonpregnant women. A *t* test found the gestational reduction of cumulative blood pressure mean level to be statistically significant, even though the values tend to increase during the second and third trimester of pregnancy.

The frequency distribution analysis is illustrated in Table III. The frequency densities document the overall distribution of both the systolic and diastolic blood pressure values as being consistently different in pregnant women as compared with nonpregnant women. The dissimilarity applies to the entire set of descriptive parameters, that is, the median, mode, extremes, range, variation coefficient, 97th percentile, and numerous-

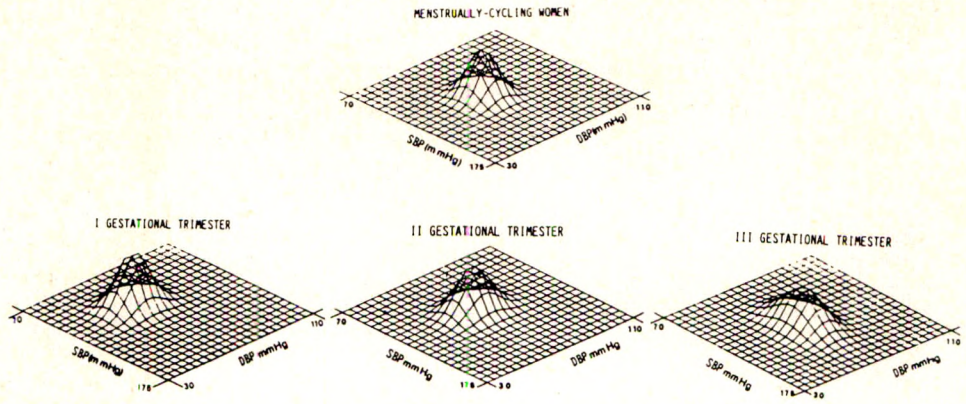


Fig. 1. Bivariate representation of the gaussian distribution found in cumulated values that constitute 24-hour systolic *(SBP)* and diastolic *(DBP)* blood pressure pattern in nonpregnant and pregnant women.

ness of constitutive frequencies. The class repartition documents that the population of blood pressure values is differently composed in pregnant women as compared with nonpregnant women. A median test found the differences in distribution to be statistically significant, even though the medians tend to change during the various trimesters of pregnancy.

The gaussian analysis of the systolic and diastolic blood pressure cumulative distributions is depicted in Fig. 1. The bivariate plots document that a gaussian curve is detectable in both populations of cumulative blood pressure values in pregnant women and nonpregnant women. The gestational systolic and diastolic blood pressure values, however, cover a narrower dispersion in their variability around the mean. A bivariate discriminant analysis found these differences to be statistically significant ($p < 0.001$) within their distributive normality.

Macroscopic temporal biometry. The analysis of time-qualified mean levels and their reference limits is displayed in Table IV. The estimates document that the time-qualified means for both the 24-hour systolic and diastolic blood pressure patterns are consistently different in pregnant women as compared with nonpregnant women. The gestational time-qualified dispersion of values is also peculiar. A t test found the time-qualified decrease of gestational blood pressure values to be statistically significant at each time point (significance ranging from $p < 0.01$ to $p < 0.001$).

More details on the intertime variability of the 24-hour blood pressure patterns are given in Table V. The estimates indicate that the 24-hour mean level of both the 24-hour systolic and diastolic blood pressure patterns is noticeably reduced in pregnant women as compared with nonpregnant women. The differences in time-qualified variability are also well demonstrated by the variation coefficient. A t test found the gestational decrease of 24-hour systolic and diastolic blood pressure mean level to be statistically significant ($p < 0.001$ in each trimester).

Microscopic temporal biometry

Cosinor analysis. The analysis of rhythm properties and their reference limits is reported in Table VI. The p values indicate that the sinusoidality testing validated the occurrence of a circadian rhythm for the 24-hour systolic blood pressure and diastolic blood pressure patterns in both pregnant women and nonpregnant women. However, the gestational systolic and diastolic blood pressure circadian rhythms exhibit a less pronounced mesor and a greater amplitude of oscillation. The first trimester of pregnancy is, additionally, characterized by a consistent shift of the acrophase. The parameter test found the mesor decrease in the gestational blood pressure circadian rhythm to be statistically significant, even though the parameter tends to increase during the second and third trimester of pregnancy.

Cosint analysis. The analysis of baric impact and its reference limits is summarized in Table VII. The estimates indicate that the baric impact of both the systolic and diastolic blood pressure circadian rhythms is reduced in pregnant women as compared with nonpregnant women. A t test found the gestational decrease of baric impact to be statistically significant.

Comment

An unanswered question relates to the reason(s) why blood pressure changes so deeply during pregnancy. To answer this question it is mandatory to explore blood pressure in its naturally occurring changes, taking into consideration that the physiologic arrangement is characterized by a within-day variability.[5-7]

We are aware that an approach to the 24-hour blood pressure patterns may be informative if the time series are analyzed by appropriate descriptive statistics. Thus, the statistical approach to the gestational 24-hour blood pressure patterns was made in this study in the most extensive way possible.

The first approach was the macroscopic cumulative biometry, which was performed with the scope of es-

Table VI. Reference limits for circadian rhythm of systolic and diastolic blood pressure in nonpregnant and pregnant women

Group	Blood pressure	p	Mean (90% TL)		
			Mesor (mm Hg)	Amplitude (mm Hg)	Acrophase (hr:min)
Nonpregnant women					
	Systolic	<0.001	117 (107; 126)	5 (4; 6)	14:28 (13:44; 15:12)
	Diastolic	<0.001	70 (60; 79)	2 (1; 3)	13:36 (12:04; 15:08)
Pregnant women					
First trimester	Systolic	<0.001	103* (75; 130)	9† (5; 13)	20:12† (18:40; 21:40)
	Diastolic	<0.001	64† (45; 82)	8† (2; 13)	21:12† (19:12; 23:08)
Second trimester	Systolic	<0.001	107* (88; 125)	9† (7; 12)	15:44‡ (14:04; 17:20)
	Diastolic	<0.001	67† (39; 94)	7† (1; 12)	15:12* (11:32; 18:48)
Third trimester	Systolic	<0.001	106† (60; 143)	8‡ (2; 13)	13:32§ (10:00; 17:00)
	Diastolic	<0.001	66† (47; 84)	6‡ (3; 8)	14:08§ (12:00; 16:12)

TL, Tolerance limits; *p*, significant level for the statistical validation of circadian rhythm.
*p = 0.02 by parameter test in contrast with nonpregnant women.
†p = < .001.
‡p = <0.01.
§Not significant.

Table VII. Reference limits for baric impact caused by circadian rhythm of systolic and diastolic blood pressure in nonpregnant and pregnant women

Group	SBP (mm Hg/24 hr)		DBP (mm Hg/24 hr)	
	Mean	90% TL	Mean	90% TL
Nonpregnant women	27,807	(22,794; 32,819)	17,092	(13,200; 20,975)
Pregnant women				
First trimester	25,806*	(21,771; 29,840)	16,763*	(12,901; 20,624)
Second trimester	24,331†	(19,946; 29,215)	16,099‡	(11,757; 20,440)
Third trimester	25,499‡	(20,279; 30,618)	15,913‡	(13,230; 18,595)

TL, Tolerance limits; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure.
*Not significant by *t* test in contrast with nonpregnant women.
†$p < 0.001$.
‡$p < 0.05$

timating the overall distribution of both the 24-hour systolic and diastolic blood pressure patterns. The analysis invariably documented a less pronounced mean level in gestational blood pressure. The frequency analysis, in turn, revealed that blood pressure values in pregnancy are consistently shifted toward the left side of the distribution. The discriminant analysis clarified that such a shift is statistically significant. The shift notwithstanding, the gestational blood pressure values appear to be normally distributed, authorizing the approach to their reference limits via the parametric statistics. Accordingly the reference limits computed in our study by the statistical correction of *t* distribution appear to be appropriate for deciphering the normality in the gestational 24-hour blood pressure pattern.

The second approach was the macroscopic temporal biometry performed to quantify the distribution of the gestational 24-hour blood pressure pattern around the clock. The time-qualified measures of central distribution documented that gestational blood pressure values show a homogeneous decrease in mean levels causing the temporal profile to reach a less pronounced height. The lower level of the within-day curve suggests that pregnancy acts to modify the physiologic properties of blood pressure in their global structure. This is the reason why the time-qualified standard limits reported in our study may be regarded as appropriate references for the gestational blood pressure monitoring.

The third approach was the microscopic temporal biometry that provided an unambiguous interpretation

of the bioperiodicity of the gestational 24-hour blood pressure pattern. Basically, the cosinor method documented that the blood pressure circadian rhythm shows peculiar properties in pregnant women. The rhythmometric changes manifested themselves during the first trimester; the blood pressure fluctuation took place late in the evening with a delay of about 6 hours. However, the acrophase tends to advance timing, the orthodox phase being adjusted since the second trimester of pregnancy. It is important that the blood pressure circadian rhythm in pregnancy was invariably seen to show a consistent reduction in mesor, unequivocable expression of a structural interaction with the entire pattern. The reference limits of this adjustment are clearly computed in our study.

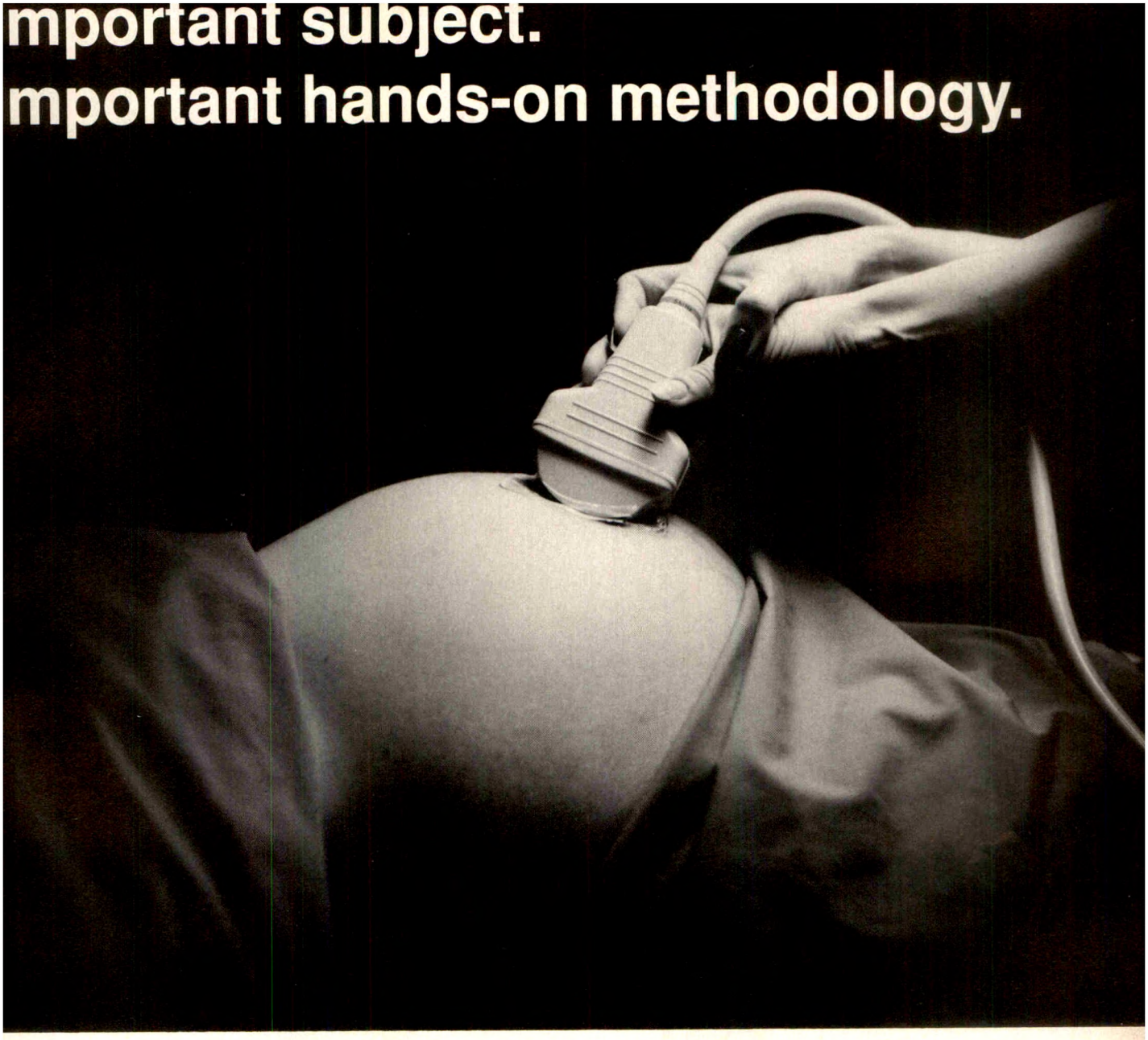
The last approach in the microscopic temporal biometry was the cosint analysis, which documented the gestational 24-hour blood pressure pattern to change in one of the most important physiologic attributes. Because the baric impact is frankly reduced, it can be concluded that pregnancy globally reduces the blood pressure load during the 24-hour span. The standard limits of this reduction are clearly visible in our study.

It must be stressed that the physiologic properties of biologic rhythms have a meaning from a functional point of view. With respect to this, it is important to remember that the mesor is the measurable expression of the tonic level for a given periodic function. The amplitude and acrophase, in turn, are the quantitative and temporal estimates of the phasic activity for a given rhythm. When the mesor varies in magnitude, its change may be seen as a quantitative modulation of the tonic property. When the amplitude changes in extent, its variation can be regarded as a quantitative modulation of the phasic activity. Last, when the acrophase rotates in timing its shift may be considered a temporal readjustment of the phasic periodicity. Translating this into the present findings means that the gestational 24-hour blood pressure pattern changes prominently in its tonic regulation. The readjustment of the blood pressure circadian tone is the mechanism for which pregnancy is able to reduce the baric impact, providing concrete protection to arterial vasculature.

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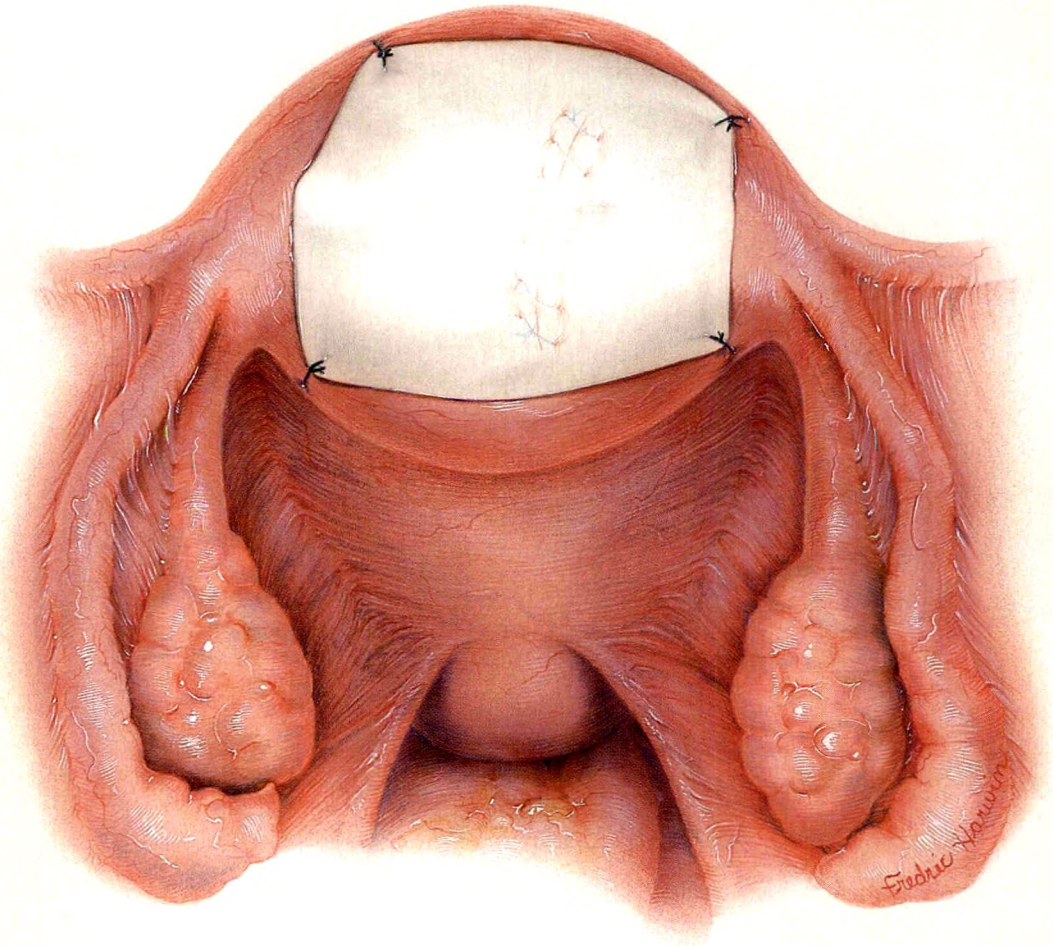
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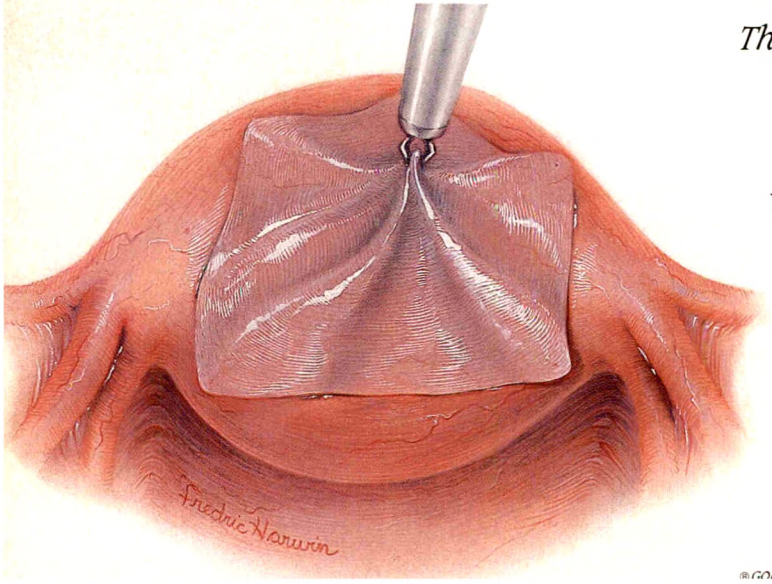
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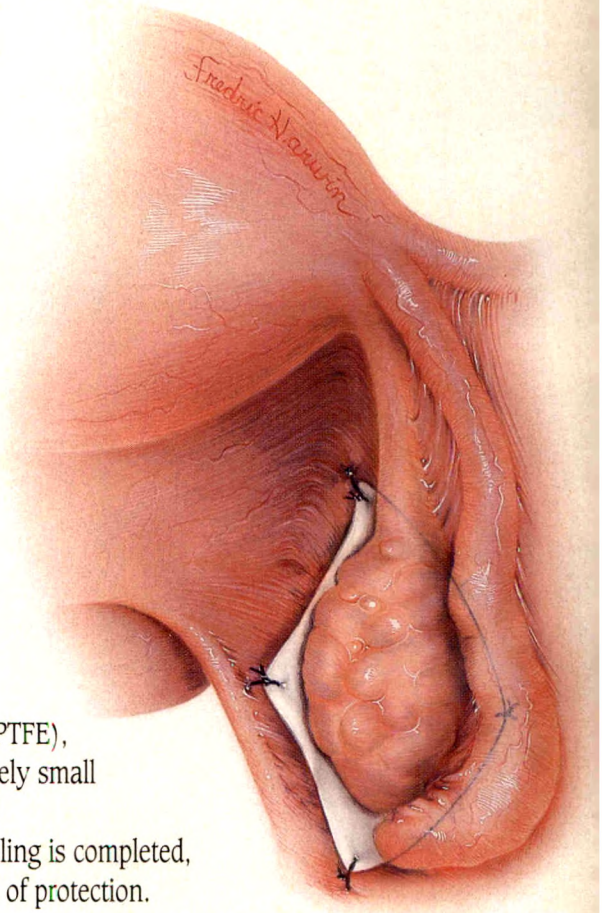




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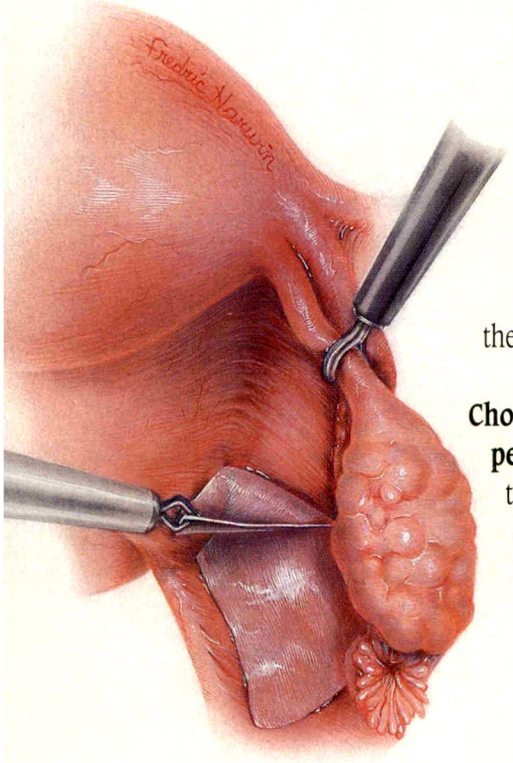


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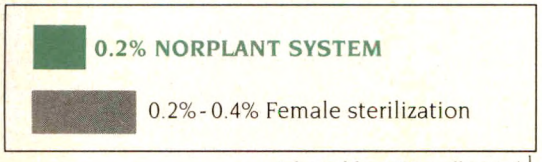


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NORPLANT® SYSTEM

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Lasts five years…yet is reversible

BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See **Warnings** section and Full Prescribing Information.

NURSING MOTHERS: See **Warnings** section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis, and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin, levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

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Derivation of new mathematic formulas for determining whether a D-positive father is heterozygous or homozygous for the D antigen

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OBJECTIVES: This article derives new mathematic formulas to calculate the probability that the husband of a D-negative woman is heterozygous for the D antigen.
STUDY DESIGN: Current published formulas and tables that estimate this probability are biased in that they incorrectly assume that the father is randomly selected from the general population. With Bayes' theorem, new formulas were derived to eliminate this bias.
RESULTS: The probability of the husband being homozygous or heterozygous for the D antigen can be expressed as a function of the number of previous D-positive children. If he has any D-negative children, he is an obligate heterozygote.
CONCLUSION: Previously published formulas and tables that estimate the likelihood of the father being heterozygous or homozygous at the D locus should no longer be used in the obstetric setting. (Am J Obstet Gynecol 1992;166:61-3.)

Key words: Erythroblastosis fetalis, Rh-Hr blood group system, Rh isoimmunization, hemolytic disease of the newborn

In spite of the success of Rh immune globulin in reducing the incidence of sensitization to the D antigen, anti-D still develops in D-negative women of childbearing age.[1] This sensitization may result from failure to receive Rh immune globulin during a previous pregnancy, failure of Rh immune globulin to prevent sensitization when it is given, or from previous transfusion of D-positive blood.

Numerous textbooks[1-9] state that when anti-D develops it is important to know whether the woman's mate is homozygous or heterozygous at the D locus. The reason for determining this is that a homozygous D-positive father will produce only D-positive offspring, whereas a heterozygous father has a 50% chance of producing a D-negative offspring in whom hemolytic disease of the newborn cannot develop as a result of anti-D. The main use of this knowledge is in advising a couple as to the risk that a future baby will have severe hemolytic disease of the newborn and in deciding about permanent sterilization or artificial insemination from a D-negative donor.[6] Others[5] state that knowledge of whether the father is heterozygous or homozygous for the D antigen has prognostic value in the patient who is already pregnant.

Unfortunately, the D antigen cannot be detected directly. Therefore determining the zygosity of the D-positive father is done indirectly by phenotyping the father for the C, c, E, and e antigens, which are closely linked to the D locus. Published formulas or tables from several standard textbooks,[1, 4-7] are then used to determine the zygosity of the D antigen. During a previous study of hemolytic disease of the newborn,[10] however, it became obvious that these formulas give biased results (subsequent text).

Specifically, the widely used formulas and tables incorrectly assume that the person being tested is selected randomly from the population. In reality, testing is performed only on husbands of D-negative women who have been sensitized to the D antigen. As noted, this situation usually occurs as a result of the couple's previously having had D-positive children. As a result, the prospective father being tested is more likely to be homozygous at the D locus than one randomly selected from the population. Similarly, if the prospective father has had even a single D-negative child, he is an obligate heterozygote. Any further testing of the father is not only unnecessary but potentially misleading.

Only a minority of authors[8, 9] recognize this bias, and they fail to give any estimate as to how large it is or what can be done mathematically to eliminate it. As noted, however, the vast majority of authors[1, 4-7] fail even to recognize any bias with currently used methods.

It is the purpose of this article to derive simple mathematic formulas and tables that will provide an unbiased estimate of the likelihood of a prospective father being homozygous or heterozygous at the D locus, on the basis of the number and Rh(D) type of previous

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Table I. Probability of heterozygosity at D locus in whites for given Rh phenotype and *n* previous D-positive offspring*

Phenotype	*n = 0*	*n = 1*	*n = 2*	*n = 3*	*n = 4*	*n = 5*
CcDe	0.90	0.82	0.69	0.53	0.36	0.22
CDe	0.090	0.047	0.024	0.012	0.006	0.003
cDEe	0.90	0.82	0.69	0.53	0.36	0.22
cDE	0.13	0.070	0.036	0.018	0.009	0.005
CcDEe	0.11	0.058	0.030	0.015	0.008	0.004
cDe	0.94	0.89	0.80	0.66	0.49	0.33

*If the father has had any previous D-negative children, then the probability that he is heterozygous for the D antigen is 1.

Table II. Probability of heterozygosity at D locus in blacks for given Rh phenotype and *n* previous D-positive offspring*

Phenotype	*n = 0*	*n = 1*	*n = 2*	*n = 3*	*n = 4*	*n = 5*
CcDe	0.41	0.26	0.15	0.080	0.042	0.021
CDe	0.19	0.10	0.055	0.028	0.014	0.007
cDEe	0.37	0.23	0.13	0.068	0.035	0.018
cDE	0.01	0.005	0.003	0.001	<0.001	<0.001
CcDEe	0.10	0.053	0.027	0.014	0.007	0.003
cDe	0.54	0.37	0.23	0.13	0.068	0.035

*If the father has had any previous D-negative children, then the probability that he is heterozygous for the D antigen is 1.

children and the presence of C, c, E, and e antigens in the prospective father.

Derivation of formulas

Only minimal assumptions will be made. For convenience, "father" shall refer to the man for whom it is desired to know whether he is homozygous or heterozygous for the D antigen. The term "mother" shall refer to his mate, who is D-negative and has anti-D in the serum. Only previous offspring by the father and a D-negative mate (she may or may not be the father's current mate) are considered in the formulas. The father will be assumed to be D positive (the formulas do not apply to D^u fathers, and if the father is D negative, there is no concern of hemolytic disease of the newborn as a result of anti-D because there can be no D-positive children). Crossover in the Rh system will be ignored.

Let i = the phenotype of the father at the C, c, E, and e loci, (P(DD | *i*) = the probability that a person randomly selected from the population is homozygous for D, given that he is D positive and has phenotype *i*, and P(Dd | *i*) = the probability that a person randomly selected from the population is heterozygous for D, given he is D positive and has phenotype *i*. Obviously (Dd | *i*) = 1 − P(DD | *i*)

The above quantities are those currently in use, and as noted above, give a bias when the father is known to have had previous D-positive offspring.

We will now use Bayes' theorem[11] to eliminate the bias in the estimates. Let *n* = the number of D-positive children that the father has had by mating with a D-negative woman. If he has had any D-negative children, he is an obligate heterozygote regardless of his tested phenotype *i*, and the formulas derived herein are not necessary. With Bayes' theorem:

$$P(DD \mid i \text{ and } n) = \frac{P(DD|i)P(n|DD)}{P(DD|i)P(n|DD) + P(Dd|i)P(n|Dd)}$$

and P(Dd | *i* and *n*) = 1 − P(DD | *i* and *n*). With Mendel's first law, P (*n* | Dd) = $(1/2)^n$ and P(*n* | DD) = 1. Thus

$$P(DD \mid i \text{ and } n) = \frac{P(DD|i)}{P(DD|i) + P(Dd|i)(1/2)^n}$$

and P(Dd | *i* and *n*) = 1 − P(DD | *i* and *n*).

Values for P(DD | *i*) and P(Dd | *i*) have already been calculated from the gene frequencies of the Rh system and are readily available.[7]

Results

Values for the probability of the father being heterozygous at the D locus [P(Dd | *i* and *n*)] are listed in Tables I and II for whites and blacks, respectively. Note that if *n* = 0 (i.e., if the father has had no previous children), the formulas derived herein reduce to those published elsewhere,[1, 4-7] as expected. One can immediately see that for the father who has had several D-positive children, the probability of his being heterozygous decreases. For some phenotypes this is quite dramatic. For example, if a white D-positive father has the phenotype CcDe, the standard tables in use would indicate that he has a 90% chance of being heterozygous for the D antigen. According to Table I, however, if

he has had three previous D-postive children, the probability of his being heterozygous drops to 0.53. A similarly large potential error exists for whites whose phenotypes are either cDEe or cDe. For the other Rh phenotypes in whites, the presence of previous D-positive offspring still decreases the likelihood of the father being heterozygous; however, the absolute decrease is less. This result occurs because even when previous offspring are ignored or none exist ($n = 0$), the father is already probably homozygous (see Table I).

For blacks, in general, there is less likelihood that the father is heterozygous than for whites. For the phenotypes CcDe, cDEe, and cDe, the father has about a 50% chance of being homozygous for the D antigen if one uses either standard published tables or if he has had no previous children. One can see, however, that if he has had several D-positive children, the odds are more likely that he is homozygous (see Table II).

Comment

The above formulas were derived with very few assumptions (i.e., Mendel's first law, Bayes' theorem). Identical results to the formulas derived herein, however, were obtained by use of more cumbersome but well-accepted methods of genetic analysis.[12]

When using these formulas, one should keep in mind that the race of the father is the one considered in these formulas and tables. Additionally, one is assuming that the father is the true biologic father of any previous children.

At first glance, one might believe that most people do not have very many children and thus any bias introduced by ignoring them is small. This may be the case in the general population, but one must remember that this analysis applies to couples in which the mother has been immunized to the D antigen. In most cases this is the result of having had two or more previous children.[9] Thus one would expect that women immunized to the D antigen, as a rule, have had significantly more children than those among the general population.

The formulas derived in this article have the advantage that all the data should be readily available. Currently, for the immunized mother, most obstetricians should obtain a detailed history, including the blood type of previous offspring and the presence and severity of hemolytic disease of the newborn in previous pregnancies. In addition, most blood banks routinely determine the Rh type of every newborn if the mother is D negative,[7] so that in many cases there may already be a record of the Rh type of any previous offspring. (Repeat testing of the previous offspring may be necessary if previous results are not available.) If a previous fetus died in utero of hemolytic disease of the newborn, for purposes of these formulas, one can assume that the fetus was D positive.

Some authors[5,8,9] have stated that family studies may be of use in determining the zygosity of the father for the D antigen. These studies, however, may still be inconclusive and family members may not be available for testing. Therefore one should use Tables I and II as a preliminary screening device. If the probability of homozygosity is sufficiently close to 0 or 1, family studies would be unnecessary. What value is sufficiently close to 0 or 1 is a completely arbitrary decision. One should keep in mind, however, that if one is 90% certain that a father is homozygous, the probability of the next offspring being D positive is 0.95. In the unusual situation in which family members are available for testing and the formulas yield results that are not sufficiently close to 0 or 1, family studies may be justifiable.

In summary, this article has described new formulas to determine whether a D-positive father is heterozygous or homozygous for the D antigen. These formulas have eliminated the errors of the currently used formulas.[1,4-7] These previously published formulas and tables should not be used for the couple with previous offspring.

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Insulin-like growth factor I and II binding in human myometrium and leiomyomas

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OBJECTIVES: The purpose of this study is to determine if the human myometrium has receptors for insulin-like growth factors I and II and whether the concentration of these receptors is increased in leiomyomas.

STUDY DESIGN: Specific binding of iodine 125–labeled insulin-like growth factors I and II was examined in the membrane preparations of myometrium and leiomyomas obtained from 10 women with uterine leiomyomas.

RESULTS: Binding studies indicate presence of specific binding sites for both insulin-like growth factors I and II in the myometrium and leiomyoma. The concentration of binding sites for insulin-like growth factor I, but not for insulin-like growth factor II, was significantly ($p < 0.01$) higher in leiomyomas than in the myometrium. The dissociation constants for insulin-like growth factors I and II receptors in both myometrium and leiomyoma were similar.

CONCLUSION: insulin-like growth factor I, but not insulin-like growth factor II, receptors are increased in leiomyomas compared with those in myometrium, indicating that insulin-like growth factor I may play a role in the generation and/or growth of this tumor. (Am J Obstet Gynecol 1992;166:64-9.)

Key words: Insulin-like growth factors, IGF-I, IGF-II, myoma

Insulin-like growth factors (IGF) I and II are single-chain peptide hormones that have a high degree of amino acid homology with each other and with proinsulin. Originally named somatomedins because of their potent growth-promoting activity in cartilage and skeletal tissues, the IGFs are now known to act on a variety of other cells and nonskeletal tissues.[1] Although traditionally the liver has been regarded as the origin of IGFs, several nonhepatic tissues have been shown to contain immunoreactive IGFs and messenger ribonucleic acid (mRNA) for IGFs.[2, 3] Recent studies indicate the presence of mRNA for both IGF-I and IGF-II in the uterine tissue, and estrogens increase this mRNA.[4-7] In the human uterus, both IGF-I and IGF-II are expressed in myometrium and leiomyomas,[6, 7] and immunoreactive IGF-I and IGF-II have been detected in explant cultures of myometrium and leiomyomas.[8]

Estrogens have been shown to stimulate the production of several growth factors in estrogen-responsive cells.[9] There is increasing evidence that the growth factors may function as autocrine, paracrine, and endocrine mediators of estrogen-induced cell growth and differentiation.[8-11] Uterine leiomyomas occur in 25% of women of reproductive age, and growth of these tumors appears to be estrogen dependent.[12, 13] It is possible that this estrogen-induced effect may be mediated through the growth factors.

The actions of IGFs and other growth factors are initiated by their interaction with specific receptors on the target cells. This study was undertaken to evaluate the possible role of IGF-I and IGF-II in the generation or growth of leiomyomas of the uterus. Experiments were designed to determine (1) if human myometrium has receptors for IGF-I and IGF-II and (2) whether the concentration of these receptors is increased in leiomyomas.

Table I. Clinical characteristics of women with and without myomas

	With myomas	*Without myomas*
Age (yr)	38.8 ± 6.3*	38.0 ± 7.0
Parity	3.0 ± 1.8	3.7 ± 1.5
Weight (lb)	210 ± 34	163 ± 80
Percent ideal body weight	172 ± 18	130 ± 53

*Mean ± SD.

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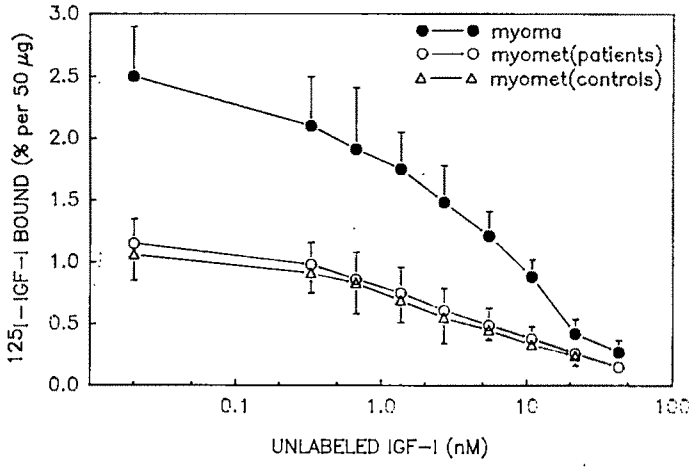


Fig. 1. Inhibition, by increasing concentrations of unlabeled IGF-I, of specific binding of [^{125}I]IGF-I to membrane preparations obtained from myoma or myometrium of women with or without myoma. Binding is expressed as specific binding in percent of total tracer added. Each *data point* is mean ± SEM for 7 to 10 individuals.

Material and methods

Tissue collection and membrane preparation. Samples of myomas and myometrium were obtained from 10 patients with uterine myomas at the time of myomectomy or hysterectomy. The ages of the women ranged from 29 to 48 years (mean 38.8 years). They were not on any hormonal treatment. All of them were multiparous and had a history of irregular menstrual cycles of 1 to 2 years' duration. Endometrial histologic studies revealed focal adenomatous hyperplasia in one patient; all the other women had proliferative endometrium. Samples of leiomyomas were obtained from the center of the tumors, to avoid contamination of myometrium. In each patient the sample was obtained from the largest of all the myomas that were present. The size of the myomas sampled ranged from 4 cm to 10 cm in diameter. For comparison, samples of myometrium were also obtained from seven women without uterine myomas who were undergoing hysterectomy during the proliferative phase of the cycle for nonendocrine problems (cervical dysplasia or uterine prolapse). Endometrial histology in all seven control women indicated proliferative endometrium. Clinical characteristics of women with and without myomas are shown in Table I. Both groups were comparable for age and parity. Body weights of patients with myoma were heavier than weights of those without myomas. Tissue samples were washed with buffer to remove any blood, immediately frozen with liquid nitrogen, and stored at −70° C until the time of preparation of membrane fractions.

Immediately before binding experiments tissue samples were chopped into small pieces with a single-edged razor blade and homogenized at 4° C in 10 mmol/L Tris hydrochloride, pH 7.8, containing 250 mmol/L sucrose and 1 mmol/L calcium chloride (Tris hydrochloride buffer).[14] Homogenization was done at a setting of 70 in a Tekmar Tissumizer (Tekmar Co., Cincinnati) with three 15-second bursts and a 1-minute cooling interval. The homogenates were centrifuged at 800 *g* for 10 minutes to remove tissue fragments and nuclei. The supernatants were recentrifuged at 35,000 *g* for 30 minutes, and the membrane pellets were washed twice and resuspended in Tris hydrochloride buffer with 0.1% gelatin. Protein concentration in the membrane preparations were determined by the method of Lowry et al.[15] with bovine serum albumin used as standard.

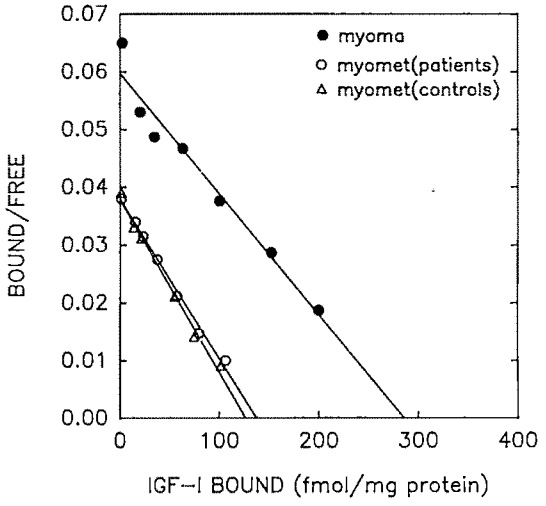


Fig. 2. Scatchard plots of specific [^{125}I]IGF-I binding to membrane preparations from myomal and myometrial tissues obtained from women with or without myoma. Each *point* represents mean of triplicates obtained from a representative individual.

Binding studies. For IGF receptor binding, membrane fractions from each myometrium and myoma

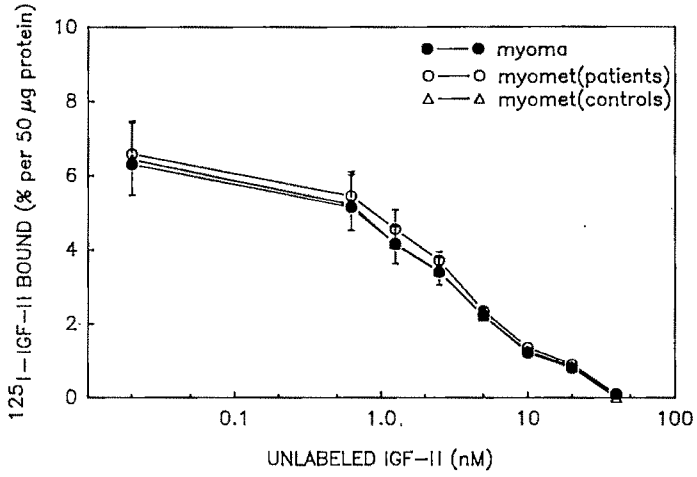


Fig. 3. Inhibition, by increasing concentrations of unlabeled IGF-II, of specific binding of [^{125}I]IGF-II to membrane preparations obtained from myoma or myometrium of women with or without myoma. Binding is expressed as specific binding in percent of total tracer added. Each *data point* is mean ± SEM for 7 to 10 individuals.

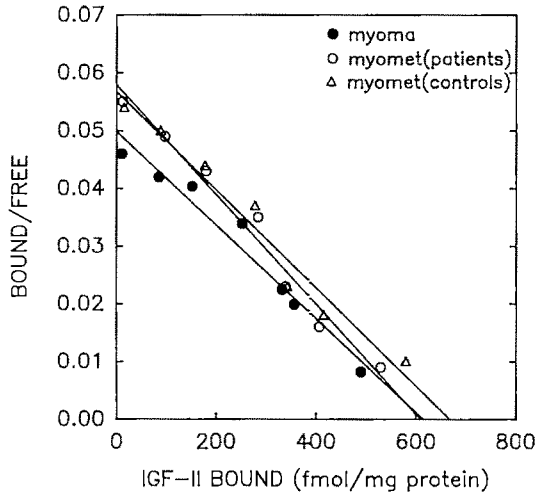


Fig. 4. Scatchard plots of specific [^{125}I]IGF-II binding to membrane preparations from myomal and myometrial tissues obtained from women with or without myoma. Each *point represents mean of triplicates obtained from a representative individual.*

specimen were incubated in triplicate with 0.01 nmol/L [^{125}I]IGF (Amersham, Arlington Heights, Ill.) in the absence or presence of seven or eight concentrations (0.33 to 43.0 nmol/L) of unlabeled IGF (Amgen Biologicals, Thousand Oaks, Calif.) in a volume of 0.2 ml Tris hydrochloride buffer with 0.1% gelatin. The incubations were carried out for 3 hours at 22° C or for 16 hours at 4° C for IGF-II and IGF-I binding, respectively. After the separation of the bound from free [^{125}I]IGF by centrifugation or filtration, specific binding data were analyzed according to the method of Scatchard with a Ligand software program to determine apparent dissociation constants and specific binding capacities. A correlation coefficient of >0.95 was obtained for all the Scatchard plots, indicating that the points form a straight line. The student *t* test was used to compare the differences in IGF binding capacities.

Specificity studies. To evaluate the specificity of IGF binding, membrane fractions of myometrium were incubated in the presence of constant amounts (1×10^5 counts/min) of [^{125}I]IGF with or without increasing concentration (0.33 to 400 nmol/L) of unlabeled IGF-I, IGF-II, or insulin.

Results

IGF-I. Specific binding of [^{125}I]IGF-I to membrane fractions obtained from myoma tissues (mean ± SE 2.5% ± 0.4% per 50 μg protein) was significantly ($p < 0.01$) higher than that to myometrial membranes obtained from the same uterus (1.15 ± 0.20). Specific binding of IGF-I to membrane fractions from myometrium of women with myomas was not significantly different from that of women without myomas (1.06 ± 0.21) (Fig. 1).

Displacement of the specific binding of [^{125}I]IGF-I to the membranes by unlabeled IGF-I was dose dependent and was similar for both myometrial and myoma tissues. Both myometrial and myoma membranes bound IGF-I with high affinity (Fig. 2). The apparent dissociation constants of IGF-I binding were similar in membranes prepared from myomas (4.9 ± 0.6 nmol/L) and myometrial tissues obtained from women with (3.9 ± 0.7 nmol/L) or without myoma (3.6 ± 0.9 nmol/L). On the contrary, the number of specific binding sites for IGF-I in myomas (299.3 ± 70.5 fmol/mg protein) were significantly ($p < 0.01$) higher than that in myometrium obtained from women with (81.8 ± 24.5 fmol/mg protein) or without (75.0 ±

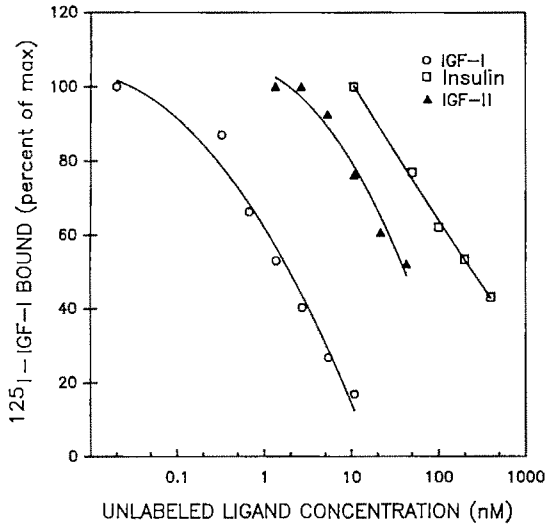


Fig. 5. Competition of increasing concentrations of cold IGF-I, IGF-II, and insulin for [^{125}I]IGF-I binding to myometrial membranes. Binding is expressed as percentage of maximum binding in absence of cold ligand. Total binding of [^{125}I]IGF-I to myometrial membranes in absence of cold ligand was 2.8%/50 μg protein. Each *point* represents mean of triplicates.

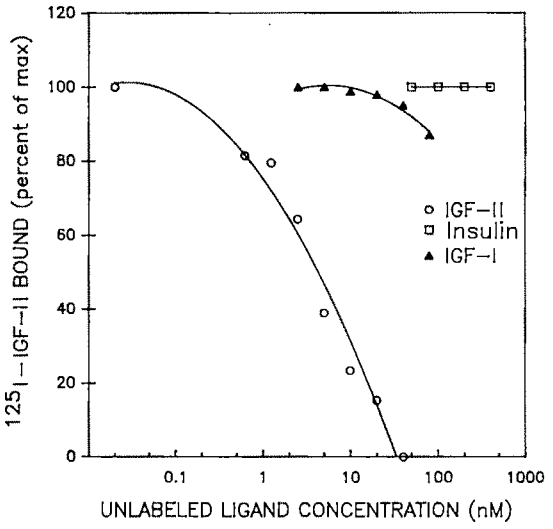


Fig. 6. Competition of unlabeled IGF-II, IGF-I, and insulin for specific [^{125}I]IGF-II binding to myometrial membranes. Binding is expressed as percentage of maximum binding in absence of cold ligand. Total binding of [^{125}I]IGF-II to myometrial membranes in absence of cold ligand was 8.9%/50 μg protein. Each *point* represents mean of triplicates.

27.5 fmol/mg protein) myoma. There was no correlation between IGF-I binding and the size of the myomas.

IGF-II. Specific binding of [^{125}I]IGF-II to membrane fractions was similar in myoma (6.58% ± 0.91% (±SE)/50 μg protein) and myometrial membranes obtained from women with (6.31 ± 0.84) or without (6.43 ± 1.11) myoma (Fig. 3). Displacement of the specific binding of [^{125}I]IGF-II to the membranes by unlabeled IGF-II was dose dependent and was similar for myometrial and myoma tissues. Scatchard analysis of the binding indicated a single class of high-affinity binding site in both myoma and myometrium (Fig. 4). The apparent dissociation constants of IGF-II binding were similar in membranes prepared from myomas (4.26 ± 0.41 nmol/L) and myometrial tissue obtained from women with (3.95 ± 0.68 nmol/L) or without (4.15 ± 0.90 nmol/L) myomas. In contrast to IGF-I, specific IGF-II binding sites are not significantly different between myoma (528.1 ± 68.7 fmol/mg protein) and myometrium with (562.3 ± 71.4 fmol/mg protein) or without (588.4 ± 94.5 fmol/mg protein) myoma.

Specificity studies. IGF-I was more potent than IGF-II in displacing [^{125}]IGF-I binding to myometrial membranes, whereas insulin demonstrated considerably lower inhibitory potency (Fig. 5). IGF-II was able to displace [^{125}I]IGF-II binding to these membranes in a dose-dependent manner. There was only slight displacement of [^{125}I]IGF-II observed with very high concentrations of IGF-I and no displacement by insulin (Fig. 6). These observations are in accordance with the general properties of IGF-I aand IGF-II receptors.

Comment

Even though uterine leiomyomas are one of the most common gynecologic tumors, little is known about their origin or what stimulates their growth. Townsend et al.[16] observed that myomas are derived from growth and proliferation of a single smooth muscle cell. In the current study we have demonstrated the presence of specific binding sites for both IGF-I and IGF-II in the human myometrium and leiomyomas. Presence of increased numbers of binding sites for IGF-I, but not IGF-II, in leiomyomas compared with myometrium indicates that IGF-I may play a role in the generation or growth of this tumor.

This is the first demonstration of IGF-II binding in leiomyomas and myometrium. There has been only one previous report on IGF-I binding in leiomyomas. Our observation of an increased number of binding sites for IGF-I in myomas is in agreement with this report.[17] We have made an additional observation that there is no significant difference in IGF-I binding sites in myometrial tissues obtained from women with or without myoma. The other growth factor that has been studied in myomas is epidermal growth factor.[17,18] EGF was not increased in myomas in these previous studies.

Insulin-like growth factors are known to bind with high affinity to two types of cell-surface receptors: type I, which generally binds IGF-I preferentially over IGF-II and shows a low affinity for insulin, and type II,

which binds IGF-II with high affinity, has negligible binding affinity for IGF-I, and has no affinity for insulin.[19, 20] In the current study we examined the specificity of IGF-I and IGF-II binding to the myometrial membranes. Binding of both IGF-I and IGF-II was specific to their receptors, as revealed from competitive inhibition of specific [^{125}I]IGF by various unlabeled ligands.

Many cell types have been demonstrated to produce IGF-binding proteins, and these binding proteins may modulate IGF action locally in various tissues.[21, 22] At least three IGF binding proteins have been sequenced and their complementary deoxyribonucleic acid cloned, and human endometrial cells have been found to secrete IGF binding proteins.[10, 21] To eliminate possible interference by these soluble IGF binding proteins in our IGF receptor assay, the membrane preparations used in these assays were washed several times before they were resuspended in the assay buffer. Using Western ligand blotting methods,[23] we were unable to detect the presence of IGF binding proteins in these membrane preparations (data not shown). Therefore the IGF binding in our assay system indeed represents IGF binding sites in these tissues.

The tendency of uterine myomas to arise and grow during reproductive years and regress after menopause indicates that estrogen could be one of the factors involved in its pathogenesis and growth. This is further supported by the fact that the size of the leiomyomas regress when estradiol levels are suppressed with long-acting gonadotropin-releasing hormone treatment.[24] A significantly higher concentration of estrogen receptors has been observed in the leiomyomas than in the adjacent normal myometrium.[25] Previous studies indicate that both IGF-I and IGF-II are expressed in uterine tissues and are regulated by estrogen.[5] Murphy et al.[5] observed that administration of estradiol significantly increased the expression of mRNA for both IGF-I and IGF-II in rat myometrial and stromal cells. In addition, IGF-I and IGF-II ribonucleic acid has been demonstrated in human leiomyomas.[7] It is possible that estrogen stimulation may enhance expression of both IGF-I and IGF-II in the myometrial tissue. The demonstration of binding sites for both IGF-I and IGF-II indicates an autocrine or paracrine role for these growth factors in uterus. Because there was an increase in myoma binding for IGF-I but not for IGF-II compared with myometrium, it is possible that IGF-I, but not IGF-II, may be mediating the estrogen-induced cell proliferation in uterine myomas and play a role in the generation and growth of leiomyomas of the uterus.

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Impairment of counterregulatory hormone responses to hypoglycemia in pregnant women with insulin-dependent diabetes mellitus

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Intensive insulin therapy directed at elimination of hyperglycemia is advocated during pregnancy in women with insulin-dependent diabetes mellitus. Because such treatment is complicated by frequent hypoglycemic episodes, we evaluated maternal and fetal responses in nine intensively treated pregnant women with insulin-dependent diabetes mellitus during an insulin-induced, gradual, controlled fall in plasma glucose levels. In contrast to values in nonpregnant control women, reductions in glucose to 44 ± 2 mg/dl in pregnant diabetic patients failed to elicit an increase in glucagon levels. Epinephrine release during hypoglycemia was also markedly suppressed in the pregnant diabetic subjects (106 ± 32 vs 327 ± 52 pg/ml in controls, $p < 0.001$). Furthermore, the plasma glucose level at which epinephrine and growth hormone were released was 5 to 10 mg/dl lower in the pregnant women with insulin-dependent diabetes mellitus ($p < 0.05$). The basal feta heart rate remained unchanged and continued to manifest accelerations during the hypoglycemic state. We conclude that the high frequency of hypoglycemia in intensively treated pregnant women with insulin-dependent diabetes mellitus may be due in part to impaired counterregulatory hormonal responses. (Am J Obstet Gynecol 1992;166:70-7.)

Key words: Hypoglycemia, pregnancy, diabetes mellitus, counterregulation, insulin

Infants of mothers with insulin-dependent diabetes mellitus are at increased risk for fetal and neonatal complications, including congenital malformations, stillbirths, macrosomia, and birth injuries.[1] Initiation of intensive insulin therapy aimed at achieving strict glycemic control after conception has resulted in a decreased incidence of all these complications, with the notable exception of congenital malformations.[2, 3] Unfortunately, the methods used to achieve tight regulation of glucose levels have been accompanied by frequent episodes of maternal hypoglycemia.[4] In one report from our center,[4] more than one sixth of blood glucose measurements were <50 mg/dl, all women had hypoglycemic symptoms, and eight of 22 experienced severe hypoglycemia. This frequency of severe hypoglycemic episodes is higher than that reported in nonpregnant patients with insulin-dependent diabetes mellitus receiving either intensive or conventional insulin treatment.[5] Although the mechanisms responsible for the high frequency of hypoglycemia in intensively treated mothers are unknown, this phenomenon may reflect more stringent target glucose goals during pregnancy.

Previous studies in nonpregnant patients receiving intensive insulin therapy suggest that such therapy is associated with the development of suppressed counterregulatory responses to hypoglycemia[6, 7] that appear to be due to a lowering of the plasma glucose level triggering counterregulatory hormone responses.[6] Whether similar adaptations occur in pregnant women with tightly controlled insulin-dependent diabetes mellitus has not been determined. The current study was consequently undertaken to examine maternal homeostatic responses and fetal well-being during a controlled hypoglycemic stimulus with the hypoglycemic clamp technique in pregnant women with tightly controlled insulin-dependent diabetes mellitus.

Material and methods

Nine pregnant patients with insulin-dependent diabetes mellitus (27 ± 2 years old) were recruited from the Yale Diabetes Faculty Practice in the Division of

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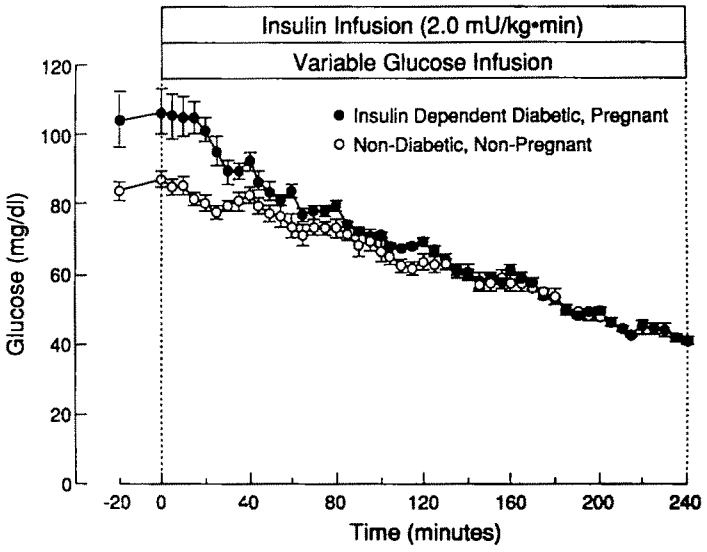


Fig. 1. Plasma glucose levels during hypoglycemic clamp studies in pregnant, intensively treated insulin-dependent diabetic women, and nonpregnant, nondiabetic control women.

Maternal-Fetal Medicine, Department of Obstetrics and Gynecology. To be eligible for study, they had to have had insulin-dependent diabetes mellitus for >1 year before pregnancy, have been treated intensively in the Diabetes in Pregnancy Program for ≥8 weeks, and have been able to achieve glycosylated hemoglobin levels <8.5% (mean ± 3 SD of nondiabetic controls). Patients were excluded if they had other hormone deficiencies or clinical evidence of autonomic neuropathy that might predispose to problems with hypoglycemia. Clinical characteristics of these women, including White's classification, are summarized in Table I. Estimated gestational age at the time of study ranged from 21 to 37 weeks (by last menstrual period and ultrasonographic assessment). The duration of diabetes mellitus in these women ranged from 3 to 22 years (average 16 ± 2 years, median 17 years).

Clinical management of diabetes during pregnancy has been previously described.[4] Briefly, pregnant diabetic women measured blood glucose levels at home approximately seven times per day (before and after each meal and at bedtime) with a reflectance meter. Accuracy of these home measurements was confirmed by weekly laboratory measurements. Insulin doses were continuously adjusted on the basis of the home glucose profiles, with the goal of fasting and preprandial levels <100 mg/dl and 2-hour postprandial levels <120 mg/dl. Rapid-or intermediate-acting insulin was administered as three or more daily injections (n = 8) or by a continuous subcutaneous insulin infusion with a portable pump (n = 1). Hypoglycemic episodes were recorded and subjectively graded as mild (responded to self-administered snack), moderate (required another person's assistance), or severe (required glucagon or intravenous glucose, usually in an emergency room setting). Obstetric management has also been previously described[4] and included antepartum biophysical and biochemical monitoring, serial ultrasonographic examinations, assessment of fetal lung maturity before elective intervention, and a policy of vaginal delivery unless intervening events necessitated cesarean section.

Seven healthy, age-matched, nonobese, nonpregnant, nondiabetic women served as controls. The mean age of these women was 27 ± 2 (range 20 to 35) and their glycosylated hemoglobin value averaged 6.5 ± 0.4. These nondiabetic women received the same hypoglycemic clamp study as the pregnant diabetics, except that the last step lowering the plasma glucose level to 45 mg/dl was omitted (because of symptoms in the nondiabetic subjects).

Written informed consent was obtained from all subjects before the study. The study protocol was approved by the Human Investigation Committee of Yale University School of Medicine.

Procedures. Studies were performed after a 10-hour overnight fast. Diabetic subjects were admitted to the Yale General Clinical Research Center on the evening before the study. Their evening dose of intermediate-acting insulin was omitted. An indwelling intravenous catheter was placed in an antecubital vein for monitoring plasma glucose, and basal insulin replacement was provided by a low-dose intravenous infusion of insulin that was adjusted every 30 to 60 minutes to achieve plasma glucose levels of ~100 mg/dl, without nocturnal hypoglycemia, as previously described.[6]

On the morning of study a second catheter was inserted into a dorsal hand vein, and that hand was then placed in a heated box (65° to 70° C) to arterialize ve-

Table I. Maternal characteristics of pregnant insulin-dependent diabetic women undergoing hypoglycemic clamp testing

Subject No.	Age (yr)	Gravidity	Parity	White's classification	Estimated gestational age (wk)	
					Initial visit	Study
1	24	3	0	FR	10	27
2	35	3	2	D	PC	31
3	29	1	0	R	10	33
4	34	2	1	FR	9	36
5	28	1	0	B	22	32
6	24	1	0	FR	9	21
7	31	3	0	FR	9	28
8	20	2	0	D	13	37
9	22	1	0	C	14	27

*Required another person's assistance.
†Required glucagon or intravenous glucose.

Table II. Characteristics of infants of insulin-dependent diabetic mothers who underwent hypoglycemic clamp testing during gestation

Subject No.	Estimated gestational age at delivery (wk)	Interval between study and delivery (days)	Method of delivery	Indication for cesarean section	Apgar scores		Weight (gm)	Neonatal complications
					1 min	5 min		
1	39	80	Cesarean section	Cephalopelvic disproportion	9	9	2892	None
2	40	59	Vaginal		8	9	3950	Hyperbilirubinemia
3	36	21	Cesarean section	Cephalopelvic disproportion	9	9	2970	None
4	39	22	Vaginal		9	9	3420	None
5	39	50	Vaginal		9	9	3870	None
6	38	114	Cesarean section	Cephalopelvic disproportion	9	9	2660	None
7	39	77	Cesarean section	Cephalopelvic disproportion	9	9	3720	None
8	39	12	Vaginal		9	9	3460	None
9	39	91	Cesarean section	Cephalopelvic disproportion	9	9	3520	None

nous blood.[8] This line was used for blood sampling during the clamp study. After a 30-minute rest period, two blood samples were obtained at 20 minute intervals for measurement of baseline glucose and hormone levels. A primed continuous infusion of rapid-acting human insulin (Novolin R, Squibb-Novo, Princeton, N.J.) was then begun; the continuous rate was 2 mU/kg/min, preceded by priming of 8 mU/kg/min for 5 minutes and 4 mU/kg/min for 5 minutes. Plasma glucose levels were determined every 5 minutes during the insulin infusion with a Beckman Glucose Analyzer II (Beckman, Fullerton, Calif.), and a variable infusion of 20% dextrose was adjusted to maintain the desired glycemic plateau. The plasma glucose level was brought to ~90 mg/dl by the end of the first 40 minutes of the study. Thereafter, plasma glucose was reduced by 10 mg/dl every 40 minutes until a plateau of 50 mg/dl was achieved. In the pregnant diabetic women the study was extended for an additional 40 minutes to allow plasma glucose to fall to 45 mg/dl.

In all pregnancies in which the estimated gestational age of the fetus was ≥30 weeks, a reactive nonstress test was obtained before the study was begun. In those fetuses <30 weeks estimated gestational age or with a nonreactive nonstress test, fetal well-being was established by a biophysical profile score of at least 8 out of 10. Fetal heart rate (FHR) monitoring was performed to assess fetal well-being during each 40-minute plateau. At the completion of the study, the FHR was monitored during reestablishment of a normal glucose level and again after lunch.

Determinations. Plasma glucose was measured in duplicate at the patient's bedside with a glucose analyzer (Beckman). The calibration of the analyzer was checked at 30-minute intervals; the coefficient of variation for the assay was <2%. Catecholamines were measured

Glycosylated hemoglobin (%)		*Mean glucose (mg/dl) in week before study*	*Hypoglycemic episodes*	
Initial visit	*Study*		*Moderate* (No./wk)*	*Severe† (No./wk)*
5.7	5.9	129	2.4	0
4.7	5.6	135	3.9	0
7.6	6.0	85	4.9	1
10.8	8.3	97	2.5	0
6.2	7.0	132	2.3	0
8.5	7.6	138	1.5	2
5.9	4.8	128	2.3	2
6.8	5.8	122	1.2	1
10.9	7.5	139	1.8	2

with a radioenzymatic assay (Amersham, Arlington Heights, Ill.). Growth hormone (Kallestad Diagnostics, Austin, Tex.), cortisol (Clinical Assays, Cambridge, Mass.), and glucagon (ICN Biomedicals, Inc., Carson, Calif.) were determined by radioimmunoassay. Human chorionic gonadotropin and prolactin were measured by immunoradiometric techniques (Serono Diagnostics, Woking, Surrey, England). Human placental lactogen (hPL) levels were determined by Smith Kline Clinical Laboratories, King of Prussia, Pa. Glycosylated hemoglobin was measured chromatographically after saline solution washing to remove the labile fraction with a microcolumn kit (Isolab, Akron, Ohio).

Analyses. The weekly mean glucose value (see Table I) represents the average of every preprandial and postprandial glucose measurement for a 1-week period before the study and comprises at least four measurements each day of the week. The plasma glucose threshold for hormone release determined during the clamp procedure was defined as the glucose level at which an unequivocal sustained increment in circulating hormone concentrations above basal levels was first observed: 75 pg/ml for epinephrine, 50 pg/ml for norepinephrine, 7 ng/ml for growth hormone, and 7 μg/dl for cortisol.[6] In each subject the mean of all control hormone values was used as the basal hormone level from which significant change was measured. To avoid the confounding effects of spontaneous growth hormone peaks, we used the mean of the four nadir values during the euglycemic phase as the baseline for defining growth hormone thresholds. The incremental value for epinephrine was chosen as one likely to be of clinical relevance,[9] and the values for growth hormone and cortisol were chosen from published definitions of normal responses to the insulin tolerance test of pituitary-adrenal axis function as previously described.[10] These definitions were used to avoid errors caused by small spontaneous fluctuations in hormone levels during the study. Because of differences in basal values during pregnancy for growth hormone and cortisol, these data are presented as the increment above basal values.

Data are presented as mean ± SEM. Comparisons between the diabetic and nondiabetic groups were made by analysis of variance with a repeated-measures design and by the Student t test. Comparisons within each group were performed by analysis of variance and paired t test. A p value <0.05 is reported as significant.

Results

Diabetes control. As shown in Table I, there was a modest variation in weekly mean glucose levels (range 85 to 139 mg/dl) in the patients during intensive treatment. At the time of the clamp study, all but one of the subjects had glycosylated hemoglobin levels in the normal range (i.e., 4% to 8.5%). As expected,[4] achievement of stringent treatment goals was associated with the common occurrence of moderate and severe hypoglycemic episodes (Table I).

Counterregulatory hormone responses. As shown in Fig. 1, basal glucose levels after the overnight fast were higher in the pregnant women with insulin-dependent diabetes mellitus than in control women (105 ± 8 vs 85 ± 2 mg/dl, $p < 0.05$). However, plasma glucose levels were virtually identical in the two groups after the initial 40 minutes of the study (Fig. 1).

As shown in Fig. 2, basal glucagon, epinephrine, and norepinephrine levels were similar in the pregnant diabetics and healthy nonpregnant control subjects. After induction of hypoglycemia, glucagon levels (109 ± 11 pg/ml, basal) in the pregnant subjects with insulin-dependent diabetes mellitus declined slightly to 91 ± 12 pg/ml (p = not significant) by the end of the study (plasma glucose 44 ± 2 mg/dl). In contrast, in the nonpregnant, nondiabetic control group glucagon initially declined but then rose from a plateau level of 75 ± 12 pg/ml to 115 ± 21 pg/ml ($p < 0.01$) when plasma glucose fell to 48 mg/dl.

In the nondiabetic women, plasma epinephrine rose from 41 ± 11 to 327 ± 52 pg/ml ($p < 0.001$) at the

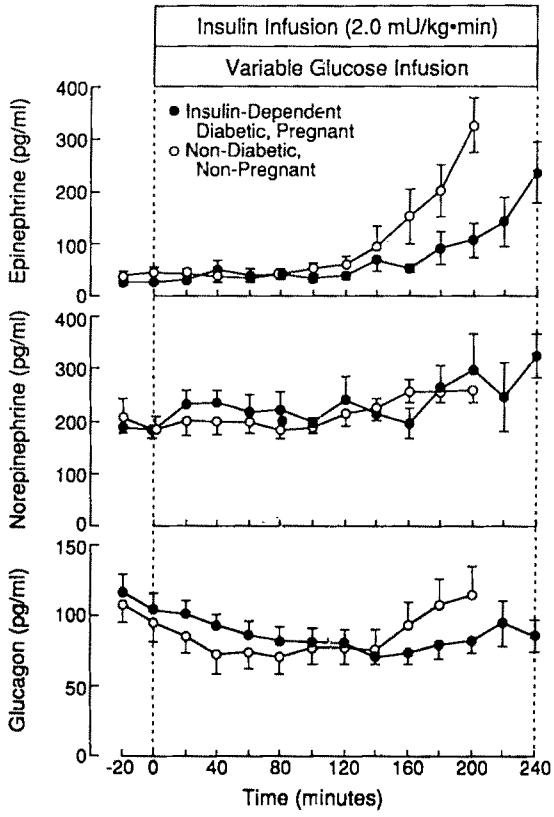


Fig. 2. Epinephrine, norepinephrine, and glucagon responses to hypoglycemia in pregnant, intensively treated insulin-dependent diabetic women, and nonpregnant, nondiabetic control women.

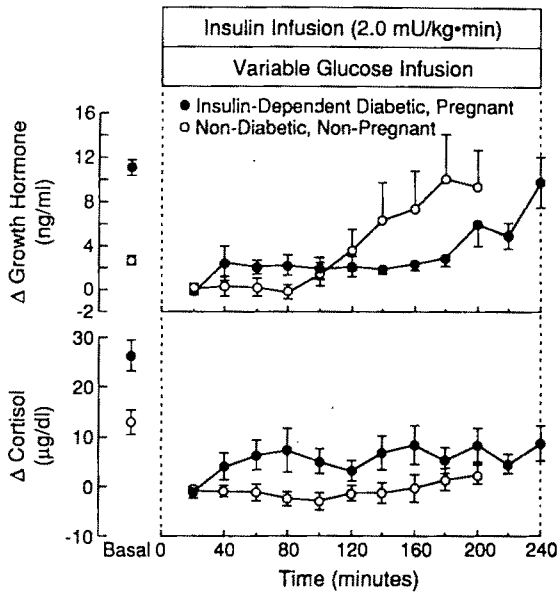


Fig. 3. Changes in growth hormone and cortisol levels from basal concentrations during hypoglycemic clamp studies in pregnant, intensively treated insulin-dependent diabetic women, and nonpregnant, nondiabetic control women.

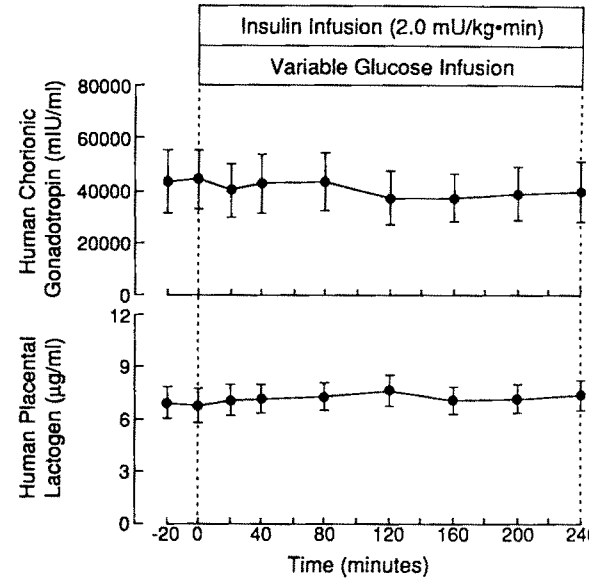


Fig. 4. Human chorionic gonadotropin and hPL levels in pregnant, intensively treated insulin-dependent diabetic women during hypoglycemic clamp studies.

Table III. Glucose threshold for counterregulatory hormone secretion

	Nonpregnant, nondiabetic controls (mg/dl)	*Pregnant diabetic subjects (mg/dl)*
Epinephrine	55 ± 3	46 ± 2*
Norepinephrine	57 ± 5	49 ± 4
Growth hormone	50 ± 1	44 ± 2†

*$p < 0.05$ versus controls.
†$p < 0.01$ versus controls.

end of the study when the plasma glucose level was 50 mg/dl. In contrast, in spite of equivalent hypoglycemia, the rise in the plasma epinephrine level in the pregnant diabetic women was blunted and delayed. When the plasma glucose level was lowered to 50 mg/dl in the pregnant women with insulin-dependent diabetes mellitus the rise in plasma epinephrine level was only one third (106 ± 32 pg/ml) of that seen in control women ($p < 0.001$). Even when the glucose level was lowered to 44 ± 2 mg/dl, the epinephrine level was still lower than that observed in controls (Fig. 2). Furthermore, the plasma glucose threshold triggering release of epinephrine was 46 ± 2 mg/dl, a value significantly less than that in control subjects (55 ± 3, $p < 0.05$) (Table II). There was no significant difference in the rise of norepinephrine in the two groups during hypoglycemia.

Basal immunoreactive growth hormone levels were elevated in the pregnant diabetic women (11.0 ± 0.7

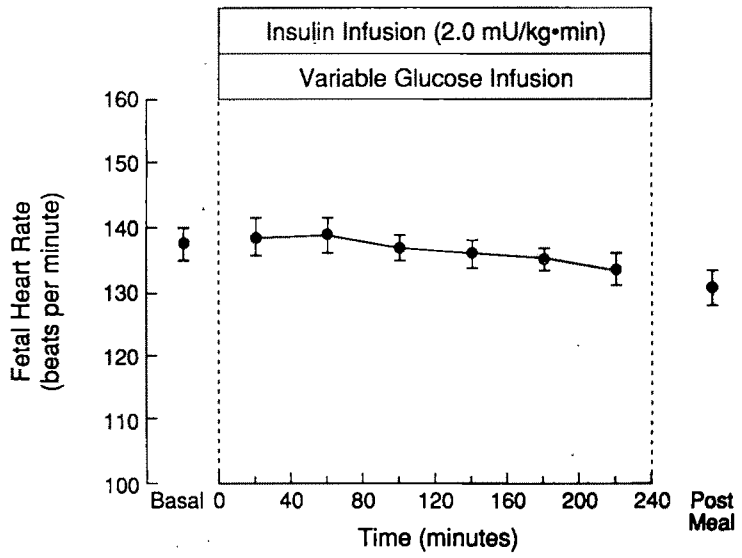


Fig. 5. FHRs from pregnant, intensively treated insulin-dependent diabetic pregnancies during hypoglycemic clamp studies.

Table IV. Glucose and hormone levels during hypoglycemic clamps in previously described nonpregnant subjects and pregnant subjects in current study with insulin-dependent diabetes mellitus

	Nonpregnant diabetic subjects		*Pregnant diabetic subjects (this study): Intensive treatment*
	Conventional treatment	*Intensive treatment*	
Glucose (mg/dl)			
Basal	92 ± 1	90 ± 1	105 ± 8
180-200 min	53 ± 1	50 ± 1	50 ± 1
220-240 min	48 ± 2	42 ± 1	44 ± 2
Epinephrine (pg/ml)			
Basal	55 ± 10	41 ± 9	26 ± 4
180-200 min	477 ± 75	138 ± 27	96 ± 29
220-240 min	690 ± 123	320 ± 64	194 ± 46
Growth hormone (ng/ml)			
Basal	13.2 ± 2.1	9.7 ± 1.4	1.0 ± 0.7
180-200 min	19.9 ± 2.3	17.4 ± 4.6	15.8 ± 1.5
220-240 min	21.2 ± 3.5	30.3 ± 6.3	19.6 ± 2.5

ng/ml) as compared with controls (2.7 ± 0.4 ng/ml; $p < 0.001$, Fig. 3). Nevertheless, the increase in growth hormone in the final 80 minutes in the nonpregnant, nondiabetic controls was greater than that in the pregnant diabetics. Additionally, the plasma glucose threshold for release of growth hormone was significantly suppressed in the pregnant diabetics (44 ± 2 vs 50 ± 1 in controls, $p < 0.01$) (Table II). Basal cortisol levels were also significantly higher in the pregnant diabetic individuals than the control women (26.3 ± 31 vs 12.9 ± 2.5 μg/dl $p < 0.01$, Fig. 3). Cortisol levels rose slightly, but insignificantly above baseline, in both groups during the study. There was no significant change in plasma human chorionic gonadotropin, hPL (Fig. 4), or prolactin during hypoglycemia.

Fetus. The baseline FHR of the eight fetuses ≥27 weeks' estimated gestational age during the control period ranged from 128 to 150 beats/min, with a mean of 138 ± 3 beats/min. Reduction in the glucose level to 45 ng/dl was associated with no significant change in the mean FHR (Fig. 5) and continued identification of FHR accelerations.

Neonatal outcome. As shown in Table III, delivery of infants occurred at an average of 38.2 ± 0.6 weeks' gestation (range 34 to 40 weeks) and followed the conclusion of the study by 63.2 ± 11.5 days (range 12 to 114 days). Five of the infants were delivered vaginally and four by cesarean section; indications for cesarean section were cephalopelvic disproportion in all four women. Average Apgar scores at 1 minute and 5 minutes were 8.7 ± 0.2 and 8.8 ± 0.2, respectively. The average fetal weight was 3385 ± 151 gm. The only neonatal complication was hyperbilirubinemia in one infant (Table III).

Comment

The detrimental effects of poor maternal glycemic control on the fetus throughout gestation[11, 12] have led to the common use of intensive insulin therapy in women with insulin-dependent diabetes mellitus in an attempt to maintain normal glucose profiles. Although improvements in metabolic control clearly diminish the incidence of fetal complications,[2, 3] it is uncertain whether the more stringent goals of achieving normal blood glucose profiles in pregnancy offers additional benefits outweighing the risk of increased fetal and maternal hypoglycemia. Cousan et al.[4] have previously reported a very high frequency of hypoglycemic events with strict glycemic control. In part, this may be due to the large doses of insulin that are required to meet target plasma glucose goals, especially in the later stages of pregnancy. Alterations in counterregulatory mechanisms may contribute as well. Nonpregnant[6, 7] patients with long-standing insulin-dependent diabetes mellitus lose the ability to secrete glucagon in response to hypoglycemia.[13, 14] Although adrenomedullary and other counterregulatory responses are sufficient to compensate for the loss of glucagon in many patients, recent studies in nonpregnant subjects with insulin-dependent diabetes mellitus[6, 7] suggest that intensive insulin therapy itself may adversely effect the magnitude and timing of the release of other counterregulatory hormones (e.g., epinephrine, growth hormone, and cortisol). Such acquired deficits in counterregulation might be an additional factor contributing to the frequent occurrence of severe hypoglycemia during diabetic pregnancies where vigorous attempts at restoring normoglycemia have been commonplace.

In this study we evaluated pregnant patients with insulin-dependent diabetes mellitus receiving intensive insulin therapy and experiencing frequent hypoglycemic episodes and demonstrated deficient secretion of glucagon and retarded and reduced release of epinephrine and growth hormone in response to a standardized hypoglycemic stimulus compared with nonpregnant, nondiabetic women. Furthermore, there was no increase in secretion of hPL during hypoglycemia in our patients. It is noteworthy that hPL levels have been shown to rise in nondiabetic pregnant subjects during hypoglycemia in some[15, 16] but not all reports.[17] Thus deficient hPL response to hypoglycemia in insulin-dependent diabetics may reflect an additional impairment attributable to diabetes or its control, a defect that represents a derangement in the fetal-placental unit rather than being solely of maternal origin.

The mechanism underlying the impaired response to hypoglycemia in pregnant women with insulin-dependent diabetes mellitus is unclear. The defect might be a result of pregnancy, the diabetic state, or the institution of strict metabolic control. An effect of pregnancy per se cannot be excluded because of our inability to perform hypoglycemic clamp studies in nondiabetic pregnancies for ethical reasons. Of the counterregulatory hormones examined, prior reports using insulin tolerance testing suggest that the growth hormone response to hypoglycemia in pregnancy may be impaired.[18-20]

Additionally, as already described, long-standing diabetes is associated with loss of the ability to secrete glucagon in response to hypoglycemia, and in some cases a reduction in the magnitude of epinephrine responses as well.[13, 14] With regard to glycemic control, institution of intensive insulin therapy is associated with impairment in counterregulatory hormone response in individuals with insulin-dependent diabetes mellitus,[6, 7] an effect associated with a reduction in the plasma glucose threshold for initiating counterregulatory responses. If we compare the epinephrine and growth hormone rise in pregnant women with insulin-dependent diabetes mellitus reported here with intensively managed nonpregnant patients with insulin-dependent diabetes mellitus reported previously,[6] a trend to greater impairment in counterregulatory hormone secretion is seen during pregnancy (Table IV). It is noteworthy that these pregnant women with insulin-dependent diabetes mellitus had lower glycosylated hemoglobin levels than in the nonpregnant subjects in the previous report, perhaps indicating better metabolic control. Alternatively, the lower glycosylated hemoglobin may be due to younger red blood cell age.

As assessed by electronic FHR monitoring, the fetuses tolerated hypoglycemia without significant changes in FHR. These findings allow some degree of reassurance for the pregnant diabetic experiencing hypoglycemic episodes, particularly in view of the clinical report of persistent severe fetal bradycardia attributed to a similar degree of maternal hypoglycemia in two patients.[21] However, the rise in maternal epinephrine levels associated with hypoglycemia (and perhaps fetal elevations of epineprhine in association with fetal hypoglycemia) did not elicit a rise in the basal FHR. It is possible insufficient amounts of maternal epinephrine crossed the placenta, that the fetus (at the estimated gestational age studied) does not respond to hypoglycemia by increasing catecholamine secretion, or that the fetus is not able to respond to epinephrine by acceleration of the FHR. More in-depth assessment of fetal well-being is required before definitive conclusions can be drawn about the impact of hypoglycemia on the fetus.

In summary, while the relative contributions of intensive insulin therapy, diabetes mellitus, and pregnancy per se is not fully delineated, this report clearly demonstrates that counterregulatory hormone response to hypoglycemia is impaired in pregnant insu-

lin-dependent diabetic women. Thus intensive metabolic regulation of such subjects is associated with frequent hypoglycemic episodes, not only because of the effect of insulin per se but also because of derangement of compensatory hormonal secretion. Importantly, the fetus tolerated this temporary hypoglycemic challenge without alteration of the FHR.

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Intrapartum amniotic fluid index and its relationship to fetal distress

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Amniotic fluid index was measured in 50 consecutive laboring women after membrane rupture. The 10th percentile of the normal range was 6.2 cm. Thirty-three women had a repeat measurement by a second observer. Although there was no systematic bias between the two observers, the limits of agreement were wide: 95% of the measurements by one observer were between 0.59 and 2.07 times those of the second. Closer agreement was observed when amniotic fluid index was low (<6.2 cm). The relationship between intrapartum amniotic fluid index and fetal distress was then investigated in a further 60 laboring women. When compared with women with a normal intrapartum amniotic fluid index, women with a low amniotic fluid index had higher incidences of fetal heart rate abnormalities during the first stage of labor (64% vs 20%, $p < 0.01$), meconium (grade II or III) at delivery (64% vs 35%, $p < 0.05$), and operative delivery for fetal distress (57% vs 17%, $p < 0.01$). Umbilical artery pH and Apgar scores were, however, similar for the two groups. Measurement of intrapartum amniotic fluid index may be an appropiate method for selecting women suitable for intrapartum amnioinfusion. (Am J Obstet Gynecol 1992;166:78-82.)

Key words: Labor, amniotic fluid infex, fetal distress

Antepartum oligohydramnios increases the risk of meconium staining, intrapartum cardiotocographic abnormalities, and operative delivery for fetal distress.[1,2] Recently measurement of amniotic fluid volume has been extended into the intrapartum period. Sarno et al.[3] reported that a low amniotic fluid index in the latent phase of labor was associated with an increased risk of cesarean section for fetal distress and 1-minute Apgar score <7. They defined a low amniotic fluid index as ≤5 cm. This figure was derived from the original studies of amniotic fluid index during pregnancy by Phelan et al.[4] A recent large study has shown this value corresponds to an amniotic fluid index <1st percentile at 37 to 43 weeks' gestation.[5] No data exist regarding the normal range of amniotic fluid index in labor.

The present study was designed to (1) define the normal range of amniotic fluid index in established labor after membrane rupture, (2) determine the reproducibility of amniotic fluid index measurements in the context of their clinical use in labor, and (3) investigate the association between a low amniotic fluid index and fetal distress.

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Material and methods

The study was conducted on the labor ward at Queen Charlotte's and Chelsea Hospital, London. The protocol was approved by the hospital Ethical Committee, and the procedure was explained to each subject before verbal consent was obtained. All the women recruited to the study were between 36 and 41 completed weeks' gestation, (confirmed by an early ultrasonographic scan) and were in established labor (cervical dilation ≥ 5 cm) with ruptured membranes and a cephalic presentation. Two groups of subjects were investigated. Fifty consecutive women, seven of whom were at ≥41 weeks' gestation, constituted the population group. Amniotomy had been performed in 23 subjects, and the membranes had ruptured spontaneously in 27. These subjects were studied by either R.A.C. or S.C.R. to determine the normal range of amniotic fluid index in labor. Sixty women, 16 of whom were ≥41 weeks' gestation, made up the study group. These subjects were selected and studied by one observer (R.A.C.) when he was resident on the labor ward. Amniotomy had been performed in 28 subjects, and membranes had ruptured spontaneously in 32. All amniotomies were performed for augmentation of spontaneous labor or for induction of labor. Women with meconium staining or fetal heart rate abnormalities were preferentially recruited into the study group in the hope of selecting subjects with oligohydramnios. A repeat ultrasonographic examination was performed by a second observer (S.C.R.) in 33 of this group to determine

Table I. Details of subjects studied

	Population group (n = 50)	*Study group (n = 60)*
Age (yr)	30 ± 5	29 ± 5
Primigravid (No.)	37 (74%)	42 (70%)
Gestation (wk)	39.7 ± 1.0	39.9 ± 1.0
Dilatation at measurement (cm)	7.7 ± 1.8	7.4 ± 1.7
Birth weight (kg)	3.43 ± 0.38	3.45 ± 0.41
Birth weight <10th percentile* (No.)	2 (4%)	3 (5%)

Figures are mean ± SD or number and percent.
*Based on data from Thomson et al.[6]

the reproducibility of intrapartum amniotic fluid index measurements. This second measurement was performed within 10 minutes of the first, and the second observer was unaware of the original results. The details of the population and study groups are shown in Table I.

Ultrasonographic examinations were performed with a portable real-time ultrasonography system (Aloka SSD 210DX) with a 3.5 MHz linear array transducer. Amniotic fluid index was measured by the four-quadrant technique described by Phelan et al.[4] Ultrasonographic measurements were not reported to the labor ward staff. All women in the study group had continuous cardiotocographic monitoring throughout labor. The time of amniotic fluid index measurement was marked on the cardiotocographic recording. The cervical dilatation and the presence and grade of meconium at the time of measurement were recorded. Meconium was graded as I to III.[7] A diagnosis of fetal distress was made by the attending medical staff on the basis of fetal heart rate abnormalities with or without scalp blood sampling.

Umbilical arterial blood gas determinations were performed within 15 minutes of delivery after double clamping of the cord. The presence of a nuchal cord together with the cord length, the presence and grade of meconium at delivery, and the 1- and 5-minute Apgar scores were recorded by the attending midwife or pediatrician. Cardiotocographs were retrospectively reviewed by one of the authors (J.A.D.S.) who was unaware of the ultrasonographic measurements. The cardiotocograph during the 30 minutes before measurement of amniotic fluid index was scored according to the criteria of Krebs et al.[8] In addition, the first- and second-stage cardiotocographic tracings were recorded as normal or abnormal (suspicious or pathologic) according to the International Federation of Gynecologists and Obstetricians classification[9] (1987), and the presence of variable decelerations was noted.

Statistical analysis. The 10th percentile for the amniotic fluid index was determined with a frequency

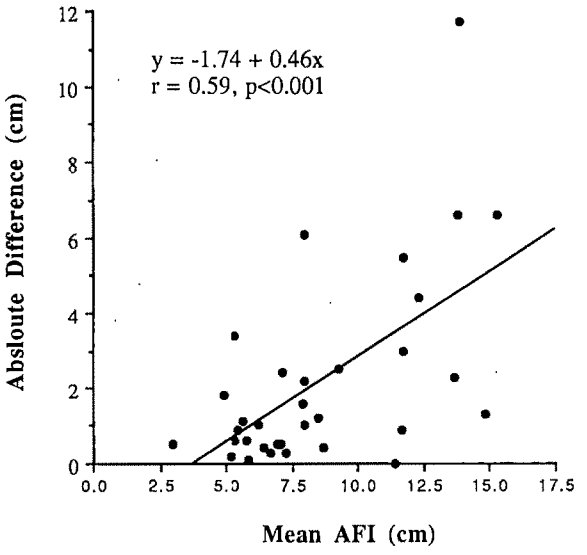


Fig. 1. Relationship between absolute difference in amniotic fluid index *(AFI)* and mean amniotic fluid index (n = 33 paired measurements).

table from the population study data. Interobserver reproducibility was assessed by the limits of agreement method.[10] Differences between the subjects with low and normal amniotic fluid index values in the study group were compared with the Student t test and χ^2 analysis.

Results

The data for amniotic fluid index from the population group were normally distributed with a mean ± SD of 9.7 ± 2.8 cm. The 5th and 10th percentiles were 3.4 and 6.2 cm, respectively.

A statistically significant correlation was found between the absolute difference and the mean of paired measurements (Fig. 1). No significant correlation was evident after log transformation, and therefore the limits of agreement were calculated on the transformed data. The mean difference was not significantly different from zero ($\log_e$ = 0.101, 95% confidence interval − 0.007 to 0.210), indicating no systematic bias between the observers. However, the limits of agreement were wide; in 95% of cases amniotic fluid index measurements by R.A.C. were 0.59 to 2.07 times those by S.C.R. Although agreement over the range of amniotic fluid index was poor, the diagnosis of a low amniotic fluid index appeared to be more reproducible: Of the nine women in which S.C.R. recorded an amniotic fluid index <6.2 cm, R.A.C. agreed in eight cases.

Of the 60 women in the study group, 14 had a low amniotic fluid index (defined as <10th percentile, i.e., <6.2 cm) at the time of measurement. The labor and birth weight details were similar in the two groups (Table II). The incidence of intrapartum complications

Table II. Pregnancy and delivery outcome in relation to amniotic fluid index in study group ($n = 60$)

Variable	*Normal amniotic fluid index (n = 46)*	*Low amniotic fluid index (n = 14)*	*p Value*
Gestation (wk)	40.0 ± 1.1	39.7 ± 0.7	NS
Dilatation (cm)*	7.2 ± 1.7	8.0 ± 1.4	NS
Labor (No.)			
Induced	14 (30%)	1 (7%)	NS
Amniotomy	23 (50%)	8 (57%)	NS
Epidural analgesia	39 (85%)	14 (100%)	NS
Birth weight (kg)	3.50 ± 0.41	3.28 ± 0.37	NS
Length of cord (cm)	61 ± 16	62 ± 19	NS
Nuchal cord (No.)	20 (43%)	6 (43%)	NS

Figures are mean ± SD or number and percent. *NS*, Not significant.
*At time of amniotic fluid index.

Table III. Intrapartum complications and neonatal outcome with normal and low (<6.2 cm) amniotic fluid index in study group

Variable	*Normal amniotic fluid index (n = 46)*	*Low amniotic fluid index (n = 14)*	*p Value*
Meconium* (No.)			
Measurement	5 (11%)	5 (36%)	<0.05
Delivery	16 (35%)	9 (64%)	<0.05
Krebs score (30 min before ultrasonography)	8.3 ± 1.5	7.7 ± 1.5	NS
Abnormal first-stage cardiotocogram	9 (20%)	9 (64%)	<0.01
Fetal blood sampling during first stage (No.)	13 (28%)	9 (64%)	<0.05
Variable decelerations during first stage (No.)	7 (15%)	8 (57%)	<0.01
Abnormal second stage cardiotocogram (No.)	22 (48%)	9 (64%)	NS
Operative delivery for fetal distress (No.)	8 (17%)	8 (57%)	<0.01
Umbilical artery pH	7.19 ± 0.10	7.20 ± 0.09	NS
Umbilical artery base excess	−9.0 ± 3.1	−9.6 ± 3.2	NS
Mean 1 min Apgar score	7.7 ± 1.4	7.9 ± 1.4	NS
1 min Apgar score ≤7 (No.)	18 (39%)	5 (36%)	NS
Mean 5 minute Apgar score	9.2 ± 0.8	9.4 ± 0.7	NS
5 minute Apgar score ≤7 (No.)	1 (2%)	0 (0%)	NS

NS, Not significant.
*Grade II or III.

and the immediate neonatal outcome in the two groups are shown in Table III. The Krebs score before amniotic fluid index measurement was similar, although significantly more women in the low amniotic fluid index group had grade II or III meconium at the time of measurement. Women in the low amniotic fluid index group were more likely to have the following: an abnormal first-stage cardiotocogram, reflecting a higher incidence of variable decelerations; fetal blood sampling during labor; an operative delivery for fetal distress; and grade II or III meconium at delivery. Although there was a trend toward an increased incidence of abnormal cardiotocograms during the second stage in the low amniotic fluid index group, this difference was not statistically significant. In spite of the higher incidence of fetal distress in the low amniotic fluid index group, the mean umbilical artery pH and base excess together with the mean Apgar scores and percentage with Apgar scores ≤7 were similar in the two groups. No infant required admission to the Special Care Baby Unit.

Comment

This is the first study to report the normal range of amniotic fluid index in established labor after membrane rupture. We chose to confine the study to women in the active phase of labor after ruptured membranes. The rationale for this was that we felt oligohydramnios and cord compression were most likely to occur at this time. The 10th percentile for intrapartum, on the basis of this consecutive series of women at term, was 6.2 cm. This is equivalent to the 2.5th percentile at 37 to

Table IV. Predictive ability of low amniotic fluid index for subsequent fetal distress

	Prevalence (%)	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
Meconium (grade II or III)	42	36	86	64	65
Variable decelerations (stage I)	25	53	87	57	85
Fetal scalp sampling	37	41	87	64	72
Operative delivery for fetal distress	27	50	86	57	83
Umbilical artery pH <7.12	20	25	77	21	80
Apgar score <7 at 1 min	20	33	79	29	83

43 weeks' gestation,[5] suggesting that the definition of intrapartum oligohydramnios should be lower than that used antenatally.

Interobserver variability of amniotic fluid index measurements was assessed by the limits of agreement method of Bland and Altman.[10] This method has been shown to be the most appropriate method of studying interobserver variation.[11] The results suggest that, over the range of amniotic fluid indexes studied, the variability of measurements was large. Rutherford et al.[12] reported the mean percentage errors for intraobserver and interoberver comparisons to be between 10% and 16%. Lower figures were reported by Moore and Cayle,[5] although these authors noted that for low volumes of amniotic fluid the percentage error was ≥20%. The mean percentage error for paired measurements in our study was 22%. This greater variability may be due in part to the lower amniotic fluid index values, but it may also reflect the true variability of measurements when applied in clinical practice. Although the overall variability was large, there was reasonable agreement between the observers in the diagnosis of a low amniotic fluid index.

The results of the current study confirm that women with oligohydramnios are at increased risk of intrapartum fetal distress (as evidenced by the presence of moderate or thick meconium staining and fetal heart rate abnormalities) and that they are more likely to require operative delivery because of this.[1, 3] The incidence of variable decelerations during the first stage of labor was particularly high (64%). Retrospective analysis showed that this was by far the commonest cardiotocographic abnormality and was the main indication for fetal blood sampling and operative delivery in this group. A comparable incidence of variable decelerations was reported by Strong et al.[13] in women with an amniotic fluid index ≤5 cm. The association between reduced amniotic fluid volume and variable decelerations is well documented.[14, 15] The pathophysiologic basis for these periodic changes in fetal heart rate appears to be umbilical cord compression.[16] Previous studies have shown that variable decelerations during labor increase the umbilical arteriovenous pH difference[17] and that the severity and frequency of variable decelerations correlate with fetal capillary pH.[18] It was perhaps surprising therefore that, in spite of the higher incidence of variable decelerations in the low amniotic fluid index group, we were unable to demonstrate any difference in umbilical artery pH or Apgar score between the two groups. This may reflect the relatively small numbers of women studied; 53 women would have been required in each group to demonstrate a difference in pH of 0.05 as being significant at the 5% level with 80% power.

The predictive value of low intrapartum amniotic fluid index for subsequent fetal distress, umbilical artery acidemia, and low Apgar score is shown in Table IV. The high prevalence of these abnormalities in the study group reflects the undoubted selection bias within the group. Amniotic fluid index appeared to be a better predictor of subsequent fetal distress than acidemia or low Apgar score. The figures for sensitivity and specificity of amniotic fluid index for the prediction of operative delivery for fetal distress are almost identical to those reported by Sarno et al.[3]

The association between oligohydramnios and fetal distress has led some workers to investigate the role of intrapartum saline amnioinfusion. Results from randomized controlled trials have shown that the procedure reduces the incidence of variable decelerations and meconium staining below the cords.[13, 19, 20] Some workers have also shown a reduction in the rate of operative delivery for fetal distress and an increase in umbilical arterial pH,[13, 19] but this was not confirmed by Owen et al.[21] Although oligohydramnios was suggested as the underlying abnormality in these studies, only Strong et al.[13] used this as the sole criterion to select women for amnioinfusion. The discrepant results reported by Owen et al.[21] may be due to the rather heterogeneous group of women studied and the apparently normal preinfusion amniotic fluid index in the treatment group (mean ± SD = 9.2 ± 3.7 cm). It would seem logical that future randomized trials of amnioinfusion should be limited to women with ultrasonographically proved oligohydramnios. Use of an amniotic fluid index <6.2 cm appears to predict, with

reasonable consistency, women at increased risk of fetal distress, and it is suggested that this may be an appropriate selection criterion for future studies of amnioinfusion.

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Colposcopy to establish physical findings in rape victims

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OBJECTIVE: Conventional rape examination protocols have been poor in yielding positive genital findings (10% to 30% typically). We report our experience with a revised protocol employing colposcopy to perform genital examinations of victims and to document findings.
STUDY DESIGN: Physical examinations were performed on rape victims seen by San Luis Obispo County's Suspected Abuse Response Team between 1985 and 1990, and the results were reviewed for this study.
RESULTS: Of 131 patients seen within 48 hours and who experienced penile penetration, 114 (87%) had positive findings. Colposcopic magnification allowed examiners to characterize these findings as acute mounting injuries, typically seen at 3, 6, and 9 o'clock on the posterior fourchette and consisting chiefly of lacerations, ecchymosis, and swelling.
CONCLUSIONS: An examination protocol that includes colposcopy may be the most reliable means to document and characterize genital findings in rape victims and to evaluate whether findings may be linked to a reported sexual assault. (AM J OBSTET GYNECOL 1992;166:83-6.)

Key words: Physical findings, rape, genital trauma, sexual assault

The difficulty of establishing physical findings in rape victims has been an accepted limitation in the medical and legal process that addresses sexual assault. While it is clear that a physical examination is an important element in patient management and that the findings of that examination can be a crucial factor in the corroboration of rape, conventional protocols have historically yielded positive genital findings in only 10% to 30% of cases.[1-3] This dearth of physical findings is compounded by the frequent absence of semen in laboratory specimens.[4, 5] Apart from circumstances surrounding the assault and the victim's subsequent behavior that might eliminate evidence, a significant incidence of sexual dysfunction in the offender has also been cited as a contributor to the lack of physical evidence.[4]

Over the last 13 years, many communities have formed multidisciplinary teams, adopting protocols to manage sexual assault victims in a more sensitive manner and to standardize the collection of evidence.[5-11] In spite of these efforts, the results of physical examinations have continued to yield low percentages of positive findings and have continued for the most part to depend on gross visualization. However, somewhat better results have been reported by authors using toluidine blue to evaluate patients experiencing penile penetration who are seen within 48 hours.[12, 13]

No published studies have evaluated the usefulness of the colposcope in the routine assessment of rape victims. Because we assumed, with others,[14] that colposcopic magnification might maximize visualization of genital findings, we established a protocol incorporating its use in 1984. We report here our experience using colposcopy to perform genital examinations in victims of sexual assault and compare our results to those using toluidine blue. We describe the findings afforded by colposcopic magnification and discuss the medical and forensic significance of this new technologic application.

Material and methods

This study was performed at the San Luis Obispo General Hospital, where for 6 years a medical team has used the colposcope in the assessment of genital trauma in rape victims. Since genital trauma in rape is typically associated with intromission[5] and generally assumed to resolve rapidly in most cases,[15] we were chiefly interested in what colposcopy could document in those victims who experienced penile penetration and were seen within 48 hours of the assault. Focusing on this population, we could also compare our results with those in published studies of protocols using toluidine blue (without colposcopy).

Examinations of 131 women who met these criteria were performed between January 1985 and December 1990, and their records were reviewed in preparing this report. All examinations were conducted by the Suspected Abuse Response Team (SART), which by interagency agreement evaluates all victims of sex crimes within the county. The physical examinations were performed by medical doctors and nurse examiners who are specifically trained in the forensic evaluation of sexual assault victims, usually with one doctor

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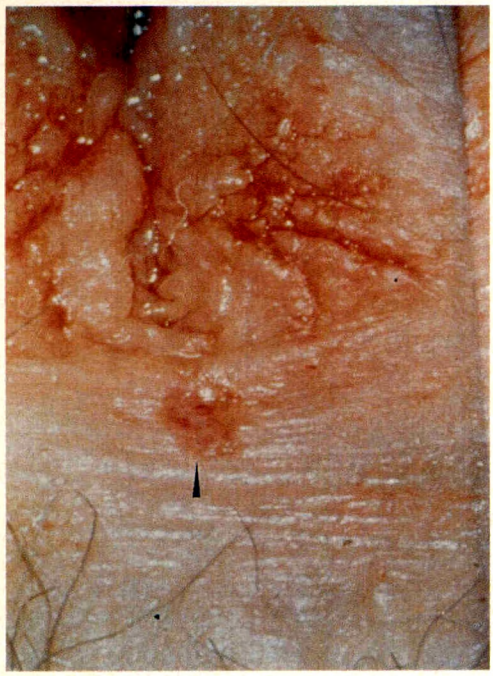


Fig. 1. Laceration on posterior fourchette *(arrowhead)* and swelling of labia minora.

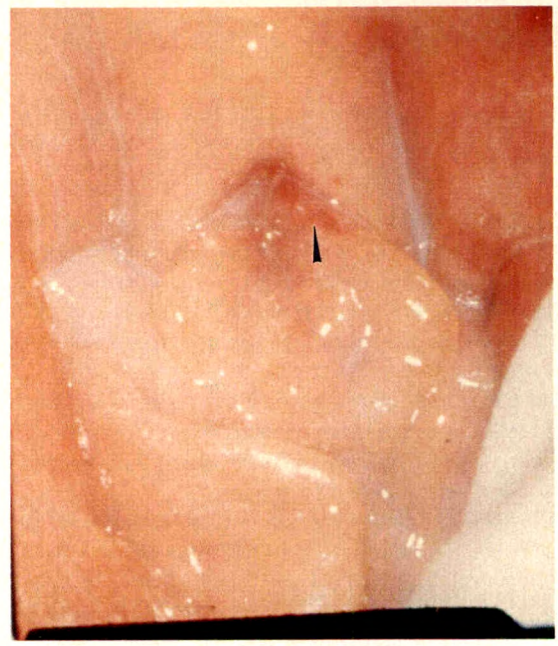


Fig. 2. Periurethral ecchymosis *(arrowhead)*.

Table I. Genital findings in rape victims by means of colposcopy

Time to examination	*No. of positive cases per total cases*
24 hr	101/117 (86%)
48 hr	13/14 (93%)
Cumulative findings	114/131 (87%)

or nurse examiner conducting the examination. Examiners have been trained to identify any abnormal findings and to distinguish between acute traumatic changes and findings more likely to have resulted from previous vaginal or vulvar infections, vulvar dystrophies, or healed obstetric lacerations or episiotomies. Colposcopic photography was used both for documentation and for interevaluator corroboration.

Once a complaint of sexual assault had been received, the victim was brought to the SART examination facility for evaluation. After appropriate consents were obtained, a detailed interview was conducted jointly by law enforcement and medical personnel, immediately followed by physical examination and collection of evidence. Photographic documentation of genital findings with a Leisegang colposcope at ×15 magnification was routinely performed. The genital examination began with gross inspection of the perineum, introitus, hymen, and approximately the distal 2 cm of the vaginal mucosa. This was followed by colposcopic assessment of the same area at ×15 magnification. Both the white light and green filter of the colposcope were used. A speculum was then inserted into the vagina. Careful evaluation of the vaginal barrel and cervix was then performed with direct visualization followed by colposcopy. After all forensic specimens were obtained, toluidine blue dye was applied to the fourchette in the usual manner.[12] This area was then reinspected grossly and colposcopically. The data were recorded on forms provided by California's Office of Criminal Justice Planning. In addition, the medical examiner dictated a summary of the history, physical findings, and recommendations. Beginning in 1986, patients with positive findings were encouraged to schedule a follow-up examination. For those who did present for reassessment, the genital examination was repeated, including colposcopy and colposcopic photography.

Results

The study population of 131 patients consisted of 113 white, 5 black, 11 Hispanic, and 2 Asian women. The mean age of the study population was 25, with a range of 13 to 85 years. With colposcopic magnification, 114 (87%) had gynecologic trauma. In addition to gynecologic trauma, 72 victims (55%) also sustained nongynecologic trauma. Only 10 victims described sexual dysfunction (defined as erectile inadequacy, premature ejaculation, or retarded ejaculation) in their attackers. All 10 suffered both genital and nongenital trauma.

With the patient examined in the lithotomy position, the predominant injuries were seen between 3, 6, and 9 o'clock on the posterior fourchette, and were concentrated in the area between 5, 6, and 7 o'clock. These

Table II. Comparison of colposcopy versus toluidine blue enhanced visualization to establish physical findings in rape victims*

Method	*No. of positive cases per total cases*	*95% Confidence intervals*
Toluidine blue		
Lauber et al.[12]	10/22 (45%)	0.242-0.658
McCauley et al.[13]	14/24 (58%)	0.382-0.778
Colposcopy	114/131 (87%)	0.812-0.928

*All values are for patients who were seen within 48 hours and who experienced penile penetration.

injuries included lacerations, abrasions, ecchymosis, and swelling. Lacerations usually occurred at the 6 o'clock position (Fig. 1). In nine instances, the injuries were extended, disrupting the fossa navicularis and producing hymenal tears. Abrasive injury to the medial aspect of the labia minora was a common accompaniment. Ecchymosis of the hymen and periurethral structures (Fig. 2) was also documented. Suturing was required for one woman suffering a perineal laceration. No patient required hospitalization because of these injuries.

Sixty-four patients returned for follow-up examination at an average of 25 days (range 4 to 54 days, excluding one patient seen at 134 days). Except in four cases where injuries were still in the process of resolution, all acute trauma had resolved by the second examination. Hymenal tears did not reunite and no scarring was observed, except in the patient who required suturing.

The results of this study are summarized in Table I. Presented are findings among subjects seen at 24 and 48 hours and the cumulative findings for the total population.

Comment

Two previous studies have used toluidine blue to document trauma in adult victims of sexual assault.[12, 13] In both, researchers showed that toluidine blue could significantly enhance visualization of genital trauma as compared with gross visualization alone. Neither study used colposcopy. We selected these for comparison, and 95% confidence intervals were constructed. As Table II shows, by this statistical method the percentages of positive findings by colposcopy are significantly greater than those in the toluidine blue studies.

Toluidine blue is a nuclear stain commonly used in gynecologic practice to detect vulvar cancer. Since normal vulvar skin contains no nuclei, it will not bind the dye. Lacerations that expose the deeper dermis will bind toluidine blue. Using this technique, some researchers have demonstrated that they could increase the number of vaginal lacerations seen grossly from one in 24 rape victims to 14 in 24 after application of toluidine blue.[13] However, this dye technique has applicability limited to the vulva and will not be taken up in areas of injury that are superficial or are beginning to reepithelialize. In our series toluidine blue did not define trauma not already seen with the colposcope. However, anticipating the need for a clear pictorial presentation of the findings in court, toluidine blue is useful to highlight injuries that a lay audience would find difficult to appreciate.

In addition to the history and initial findings, documentation of the healing process in our 64 subjects reporting for follow-up examination is important because it links the traumatic event to the injuries observed, establishing a time frame reliably associated with injury. Furthermore, documentation of the victim's anatomy at the follow-up examination effectively establishes her as her own control, against which we can evaluate the nature, extent, and cause of the injury. The performance of a follow-up examination is therefore strongly recommended; reassessment of the victim not only addresses the needs of the criminal justice system but provides more complete information about the resolution of genital injury seen in this group of patients.

The results of our study (including comparisons with the toluidine blue series) support the conclusion that colposcopy may be the best examination method yet reported for seeing genital findings associated with rape and for evaluating whether findings may be linked to a reported assault.

Only one study using the colposcope to evaluate genital changes after consentual sex exists in the medical literature.[14] Eighteen women were examined within 6 hours of intercourse. Microabrasions were seen in only two. Included in the authors' definition of trauma was increased vascularity, consisting of telangiectasia and broken capillaries; this was seen in nine subjects, and two of these women had this finding before intercourse. Of importance, none of the changes described were localized. This is in contrast to our group of rape victims, who typically have injury at the posterior fourchette. As noted in other studies, the posterior fourchette is the anatomic site of attachment to the perineal body and hence the point of greatest stress when force-

ful stretching is applied[12]; additionally, it represents the point of first contact of the penis with the vagina,[5] and the resulting trauma can be characterized as an acute mounting injury.

Now that the technology exists to assess and document the genital changes in rape victims, more work needs to be done to describe the nature, extent, and type of findings in this group. Comparison of the frequency, pattern, or severity of trauma in rape victims and in women experiencing voluntary sex may be useful, as well.

Rape is a forensic term characterizing sexual activity perpetrated against the will of the victim. The question of consent will ultimately remain with the court; nevertheless, obtaining corroborative physical data supporting the history in a time frame consistent with the alleged sexual activity is an important part of the medical assessment. The outcome of that assessment depends on an accurate examination. The rugal folds of the posterior fourchette make genital trauma difficult to see by gross visualization alone,[13] and frequently such trauma is not detected even when visualization is enhanced with toluidine blue. Our study shows that with an improved protocol, which includes use of a colposcope, examiners practiced in discriminating acute trauma from other vulvar lesions may be able to establish with greater frequency and reliability physical findings in rape victims who have had penile-vaginal contact and are examined within 48 hours.

Prompt assessment of the rape victim by a multidisciplinary team that incorporates medical examination with colposcopy may be the best way of collecting and documenting the forensic evidence in rape cases. The clarity afforded by colposcopic magnification will also allow us to further characterize genital changes associated with rape. The findings in this study support the opinion that rape victims often sustain mounting injuries. The ability to detect and document these injuries through colposcopic photography provides valuable medical and legal information.

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Natural killer cell activity from pregnant subjects is modulated by RU 486

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Natural killer cells form an integral component of the body's innate immune system. Natural killer cell activity is reduced during pregnancy, especially in the latter half. To investigate the role progesterone may play in immunomodulating natural killer cell activity during pregnancy, we evaluated the effect of RU 486 on natural killer cells isolated from pregnant subjects. Natural killer cell activity was measured with an 18-hour, Chromium 51 release, microcytotoxicity assay with K-562 cells as target cells. We demonstrated that RU 486, in a concentration range from 5 to 40 μmol/L, augmented natural killer activity threefold to fivefold over baseline. This augmentation of activity was suppressed to baseline by the addition of excess progesterone. The addition of hydrocortisone resulted in an insignificant reduction in this augmented activity. This study suggests that progesterone may play a role as an immunomodulating factor in maternal acceptance of the fetal allograft. (Am J Obstet Gynecol 1992;166:87-90.)

Key words: Natural killer cells, pregnancy, RU 486

Allogeneic immune responses are responsible for the rejection of tissues transplanted between members of the same species.[1] In viviparous reproduction, the fetus represents an allograft that is not rejected by the maternal immune system. A number of hypotheses have been proposed to explain escape of the fetus from the basic principles of transplantation immunology. These hypotheses include considering the uterus as an immunologically privileged site, decidual T-suppressor cells down-regulating the maternal immune response, placental absorption of cytotoxic antibodies, maternal production of blocking antibodies, or placental production of circulating immune suppressants.[2]

The innate immune system plays a vital role as a nonspecific first line of defense against antigenic insults. Natural killer cells form an integral component of this defense and are capable of generating a non–major histocompatibility complex restricted immune response, without prior sensitization, to a number of different target cells, including virally infected cells, transformed cells,[3-7] and, when activated to lymphokine-activated killer cells, to trophoblast.[8, 9]

Natural killer cell activity is reduced during gestation, especially in the latter half.[10-13] The physiologic mechanism that results in reduction of natural killer cell activity during gestation is currently unknown, but a number of hypotheses have been advanced to explain this phenomenon.[14, 15] Szekeres et al.[16] showed that the immunosuppressive effect of pregnancy serum is dialyzable and can be removed by pretreatment with antiprogesterone antiserum. They were also able to demonstrate a dose-dependent decrease in natural killer cell activity with the addition of progesterone.[16-18] Sulke et al.[19] and Uksila[20] were unable to duplicate these results.

RU 486 is a steroid hormone synthesized by Roussel-UCLAF that has potent antiprogesterone and antiglucocorticoid effects. It functions as a competitive inhibitor at the progesterone and glucocorticoid receptors, with a higher affinity for the former than for the latter.[21-23] We sought to determine if blocking these receptors, specifically the progesterone receptor, on lymphocytes would alter natural killer cell activity in pregnant women.

Material and methods

Natural killer cells. Forty-nine subjects were recruited from the routine obstetric clinic at Walter Reed Army Medical Center. All subjects gave informed consent, with prior approval of the Committee for Protection of Human Subjects at Walter Reed Army Medical Center. The subjects ranged in age from 17 to 43, with a mean age of 26, and had a mean gravidity of 2 and a parity of 1. Gestational age ranged from 19 to 41

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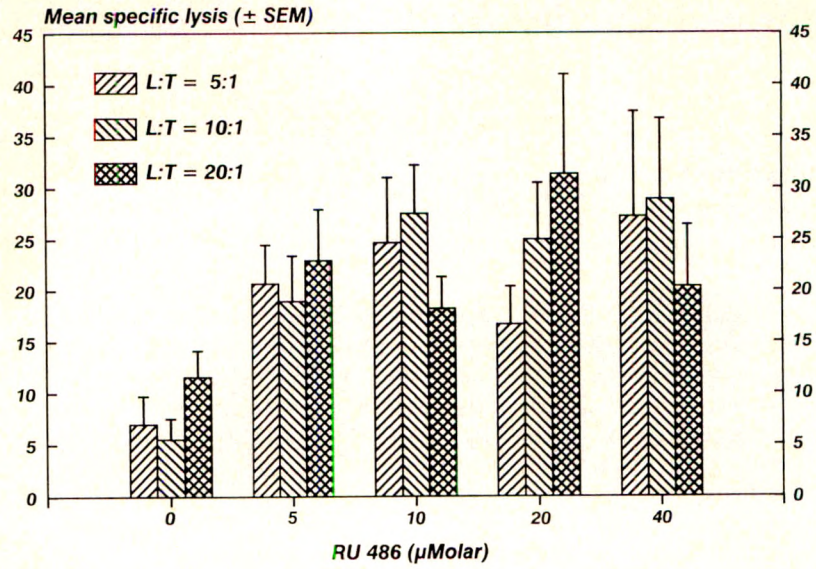


Fig. 1. Effect of RU 486 on natural killer cell activity at various lymphocyte-target cell ratios.

weeks, with a mean of 30 weeks. All subjects were healthy, were not receiving medications, and had no history of recent viral or bacterial infection.

Thirty milliliters of whole blood was obtained from each of the volunteers by standard venipuncture techniques into a heparinized test tube. This blood was diluted to 45 ml with sterile Hams F-10 medium. The mononuclear cells were separated by standard techniques with centrifugation through Ficoll-Hypaque.[24]

The mononuclear fraction was aspirated and washed two times with RPMI medium. These cells were suspended in RPMI with 10% fetal bovine serum and incubated for 1 hour at 37° C with 5% carbon dioxide in a sterile, plastic Petri dish. The nonadherent cells were collected and washed in a sterile RPMI medium. The cells were counted with a trypan blue exclusion technique to determine viability. The mononuclear cells were diluted with RPMI medium with 10% fetal bovine serum to achieve the appropriate concentration for lymphocyte/target cell ratios of 20:1, 10:1, and 5:1.

Target cells. K562 cells, a human erythroleukemic cell line, are a well-characterized natural killer cell target and were used in this series of experiments. This cell line was kept in a continuous culture in our laboratory in RPMI medium with 10% fetal bovine serum at 37° C in 5% carbon dioxide.

At the initiation of an experiment the K562 cells were harvested and washed twice. The K562 cells were labeled with 300 μCi of fresh chromium 51 for 2 hours. After two washes and counting done with a trypan blue exclusion technique to document viability, the cells were suspended in sterile RPMI medium with 10% fetal bovine serum to achieve a concentration of 10,000 viable cells per 100 μl.

Steroid hormones. The RU 486 was obtained in sterile, crystalline form from Roussel-UCLAF. It was brought into solution in 100% ethanol and titrated with RPMI medium to achieve the desired final concentration in a 30% ethanol solution. The range of concentrations of RU 486 include O (RPMI medium and 30% ethanol), 25, 50, 100, and 200 μmol/L.

Progesterone and hydrocortisone were obtained in sterile, crystalline form. These steroid hormones were brought into solution with a technique similar to the one used for RU 486.

Natural killer cell activity assay. Natural killer cell activity was determined in a ^{51}Cr release microcytotoxicity assay.[25] This assay was performed by initially pipetting 100 μl (10,000 cells) of labeled K562 cells into each well of a 96-well round-bottomed plate (Costar, Cambridge, Mass.). Then 100 μl of nonadherent mononuclear cells of varying concentrations was pipetted into each well to achieve lymphocyte/target cells ratios of 20:1, 10:1, 5:1, and 0:1 (spontaneous lysis) in triplicate. Fifty microliters of RU 486, at each of its concentrations, was pipetted into each well over the entire range of lymphocyte/target cell ratios for the initial dose-response experiments.

The same format was used to study the effect of the addition of progesterone or hydrocortisone on natural killer cells coincubated with RU 486. In this series of experiments 25 μl of 100 μmol/L RU 486 was added to each well over the entire range of lymphocyte/target cell ratios. Then 25 μl of 250 and 2500 μmol/L progesterone or 150 and 250 μmol/L hydrocortisone was added to each well over the entire range of ratios.

The 96-well plates were then incubated for 18 hours at 37° C in 5% carbon dioxide. At the conclusion of this incubation the supernatant was collected with a Titertek supernatant harvesting system (Skatron AS, Sterling, Va.), and the amount of ^{51}Cr released was de-

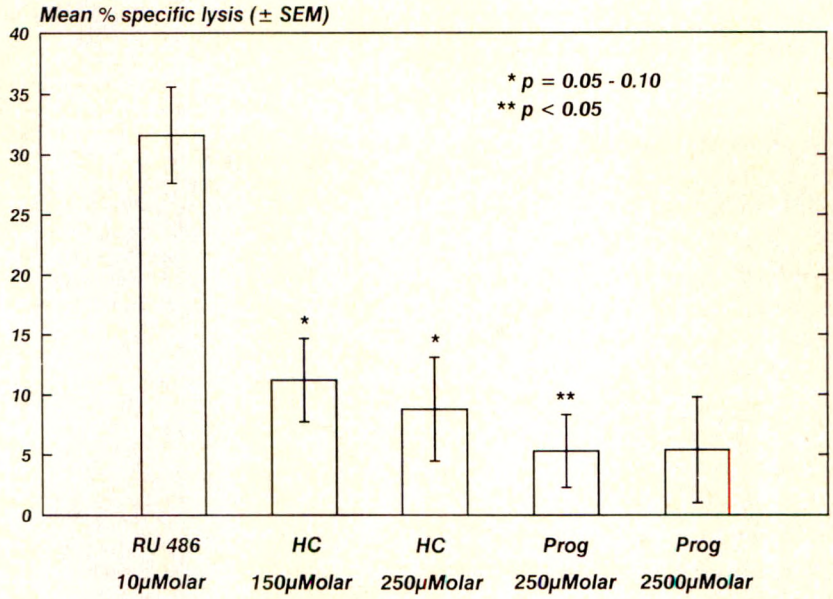


Fig. 2. Effect of hydrocortisone *(HC)* and progesterone *(Prog)* on RU 486–augmented activity. *Asterisk*, Hydrocortisone versus RU 486; *two asterisks*, progesterone versus RU 486.

termined in a γ-counter. The percent specific lysis was calculated with the following formula:

$$\% \text{ Specific lysis} = \frac{\text{Supernatant (counts/min)} - \text{Spontaneous (counts/min)}}{\text{Maximum (counts/min)} - \text{Spontaneous (counts/min)}} \times 100\%$$

Maximum counts per minute were equal to the total counts added to each well. Spontaneous lysis was equal to the counts in wells containing labeled target cells only, without nonadherent mononuclear cells, with and without the addition of hormones. Spontaneous lysis was always <30% of maximum counts per minute.

Statistics. A repeated-measures analysis of variance was used to detect any difference in natural killer cell activity created by adding RU 486. The effect of the addition of progesterone or hydrocortisone on RU 486 was analyzed with the Wilcoxon signed-rank test.

Results

RU 486 augmented natural killer cell activity threefold to fivefold above control levels at each lymphocyte/target cell ratio (Fig. 1). For each lymphocyte/target cell ratio the specific lysis at all the concentrations of RU 486 was statistically and significantly increased above control values. When the control group was excluded, there was no significant difference among the RU 486 groups.

Progesterone or hydrocortisone was added to the RU 486/lymphocyte reaction mixture from nine patients. Progesterone at a concentration of 25 μmol/L resulted in a statistically significant reduction in RU 486–augmented activity ($p = 0.008$). Hydrocortisone at a concentration of 15 and 25 μmol/L resulted in a reduction of activity ($p = 0.06$ and 0.09, respectively) (Fig. 2). Although the hydrocortisone-associated reduction did not reach statistical significance, there was insufficient power (0.55) in this series of experiments to exclude an effect.

Comment

Natural killer cells are a vital component of the innate immune system, where they primarily function in immune surveillance.[5-9] While natural killer cell activity is reduced in pregnancy, the exact mechanism producing this alteration is not known.[10-15] RU 486, a synthetic antiprogesterone, was used to further evaluate progesterone's role in immune modulation during pregnancy.

These studies revealed a statistically significant augmentation of natural killer cell activity by RU 486 over the entire range of concentrations when compared with control values. No statistical difference between the various concentrations of RU 486 were found when the control group was excluded. At the concentrations of RU 486 used in this series of experiments, we were unable to demonstrate a dose-response relationship. This suggests the need for further studies at lower concentrations to fully evaluate this effect before attributing it to an all-or-none phenomenon.

RU 486 is both an antiprogesterone and an antiglucocorticoid, with a lower affinity for the glucocorticoid receptor.[21-23] Progesterone or hydrocortisone was added to the culture wells to evaluate the specificity of the RU 486 effect. Progesterone at a concentration of 25 μmol/L significantly reduced RU 486–augmented natural killer cell activity. Hydrocortisone reduced RU 486–augmented natural killer cell activity; however, it did not reach statistical significance. These data suggest

that the primary effect of RU 486 on natural killer cell activity was through an antiprogesterone effect. Further studies with an increased number of hydrocortisone trials are necessary to clarify the role of glucocorticoids in RU 486–augmented natural killer cell cytotoxicity.

Progesterone could modulate natural killer cell activity in a direct or indirect fashion, or both. Direct modification of activity may occur by the binding of progesterone to specific natural killer cell nuclear receptors. Progesterone could alter activity indirectly by binding to receptors present in T-helper or T-suppressor cells and modifying the secretion of lymphokines by these cells. The lymphokines may then directly influence natural killer cell activity. Lymphokines having a stimulatory effect on natural killer cells include the interferons (especially inferferon gamma) and interleukin-2; prostaglandin E_2 has been shown to have an inhibitory effect.

Endometrial suppressor cells of the T-suppressor cell variety are recruited to the pregnant uterus in a hormone-dependent fashion.[26] Locally elevated levels of progesterone at the utero-placental interface may stimulate secretion of lymphokines by these T-suppressor cells. Szekeres-Bartho et al.[27, 28] demonstrated release of a suppressor factor by progesterone-stimulated lymphocytes isolated from both pregnant women and mice, which decreases natural killer cell activity in vitro. This reduction of natural killer cell activity may play a significant role in maternal acceptance of the fetal allograft.

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Prenatal diagnosis of fetal cytomegalovirus infection

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A prospective study (1988 to 1990) to evaluate the risk of fetal cytomegalovirus transmission was conducted with 1771 pregnant women; the test results of 861 were seronegative (48.6%). At each prenatal visit they were tested for serologic data and cytomegalovirus excretion in urine, saliva, and cervical secretions. If seroconversion occurred (with or without cytomegalovirus excretion), ultrasonography, amniocentesis, and cordocentesis were performed at 22 weeks' gestation; 7 cases of primary cytomegalovirus infection were investigated. In 5 cases the amniotic fluid cultures were positive; in 3 cases the fetal blood test results were positive for specific immunoglobulin M; and in 2 cases brain ultrasonography results were positive. Infection was confirmed with biopsies of fetal tissue. In two other cases, the cord blood and amniotic fluid test results were negative, and the neonates were free of infection. (Am J Obstet Gynecol 1992;166:91-4.)

Key words: Prenatal, cytomegalovirus, amniocentesis, cordocentesis, ultrasonography

Cytomegalovirus is considered to be the first cause of congenital infection.[1] Maternal infections are mostly asymptomatic; however, transplacental transmission occurs in about 50% of cases.[2] Furthermore, 90% of infected neonates show no clinical signs of infection at birth, although some will have late manifestations of congenital cytomegalovirus infection.[2] Prenatal diagnosis is therefore essential to detect those fetuses at risk of having symptoms and late sequelae. In a prospective survey seven cases of primary cytomegalovirus infection were investigated for fetal transmission. In addition to detecting specific immunoglobulin M (IgM) antibodies with cord blood tests and fetal brain echography, we emphasized virus isolation from amniotic fluid culture for antenatal diagnosis.

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6/1/32433

Material and methods

Patients. A prospective study was conducted with 1771 pregnant women; the test results of 861 were seronegative (48.6%). At each prenatal visit they were tested for serologic data and cytomegalovirus excretion in urine, saliva, and cervical secretions. If sero-

Table I. Prenatal diagnosis of cytomegalovirus infection

Case No.	*Age (yr)*	*G, P*	*Gestational age at primary infection (wk)*	*Cordocentesis*		*Amniocentesis (amniotic fluid CMV culture)*	*Brain ultrasonography result*
				IgM	*IgG*		
1	27	G2, P1	7-15	1:160	1:320	10^3 PFU/ml	–
2	29	G2, P1	8-12	+	+	10^3 PFU/ml	–
3	26	G3, P2	9-13	<1:40	1:640	10^4 PFU/ml	–
4	27	G2, P1	<9	<1:40	1:160	10^5 PFU/ml	+
5	25	G1, P0	5-10	1:20	1:20,480	10^7 PFU/ml	+
6	29	G2, P1	<9	<1:40	1:1280	–	–
7	30	G3, P1	13-18	<1:40	1:320	–	–

G, Gravida; *P*, para; *CMV*, cytomegalovirus; *PFU*, plaque-forming units.

Table II. Outcome of pregnancy and findings at termination or delivery

Case No.	Outcome of pregnancy	Fetus			Neonates (urine and saliva at birth)
		Positive CMV isolation	Positive CMV inclusions or antigen	Brain microscopic lesions*	
1	TOP	Amniotic fluid, liver, spleen	Brain, thyroid, liver, pancreas, kidneys	1, 2	
2	TOP	Amniotic fluid, liver	Brain, eye, thyroid, lung, liver, spleen, adrenal glands, pancreas, kidneys	1, 2	
3	TOP	Amniotic fluid, placenta, heart, liver, spleen, eye	Brain	1, 2	
4	TOP	Amniotic fluid, placenta, liver, spleen, eye	Brain, lung, liver, pancreas, kidneys	1-5	
5	TOP	Amniotic fluid, placenta, heart, lung, kidney, liver, spleen, eye	Brain, thyroid, lungs, liver, pancreas, kidneys, adrenal glands	1-4, 6	
6	Born				–
7	Born				–

CMV, Cytomegalovirus; *TOP*, termination of pregnancy.

**Lesion 1*, Inflammatory infiltrate; *2*, microglial nodules; *3*, focal necrosis; *4*, calcifications; 5, cortical abnormalities, 6, ependymitis.

conversion occurred (with or without cytomegalovirus excretion), ultrasonography, amniocentesis, and cordocentesis were performed at 22 weeks' gestation with informed consent from these women to detect transplacental cytomegalovirus transmission.

Serologic methods. Sera or fetal blood was titrated for IgM and immunoglobulin G (IgG) by enzyme-linked immunosorbent assay (Behring, Marburg, Germany) and for complement-fixing antibodies (antigen virion).

Cytomegalovirus isolation. Samples of urine, saliva, cervical secretions, and amniotic fluid were filtered through a 0.45 μm Minisart; 0.2 ml of the sample was inoculated in duplicate on human embryonic lung cells (MRC5 cell line, Biomerieux, Lyon, France), centrifuged at 3000 rpm for 45 minutes in macroplates (Nunclon, Roskilde, Denmark), and incubated at 37° C. After 48 hours, replication of cytomegalovirus was detected by immunoperoxidase staining with a monoclonal antibody to cytomegalovirus immediate early antigen (Biosoft, Paris, France).

The same samples were cultured on tubes to detect characteristic cytopathic effect.

Results

Seroconversion occurred in 20 of 861 seronegative pregnant women (2.3%). Seven of 20 agreed to cordocentesis and amniocentesis. The mean age of the seven patients was 27.5 years. They were all asymptomatic, except for the one who presented with clinical and hematologic signs of mononucleosis. In five cases (Table I, cases 1 to 5), amniotic fluid culture yielded a high titer of virus (up to 10^7 plaque-forming units per milliliter).

The fetal blood test results were positive in three cases (1, 2, 5) for specific IgM antibodies and negative in the two other cases (3, 4).

Brain microcalcifications were visualized in two cases (4, 5) by ultrasonography.

Our ethical committee approved the termination of pregnancies requested by these five patients. Therapeutic abortions were achieved by intraamniotic injection of prostaglandin E_2 and intravenous injection of oxytocin. Afterward the placenta was systematically manually separated and removed.

After the pregnancies were terminated, evidence of infection was confirmed either by positive culture or by demonstrating cytomegalovirus antigen or inclusions on histologic sections of various organs (brain, eye, thyroid, heart, lungs, liver, spleen, pancreas, adrenal glands, kidneys) (Table II).

In all five cases microscopic cerebral lesions consisted of inflammatory changes with perivascular infiltrates, focal areas of ependymal disruption, and glial nodules (Fig. 1, *B*). Calcified necrotic areas (Fig. 1, *A*) were observed in the periventricular region in cases 4 and 5. Microglial and neuronal cells containing cytomegalovirus inclusions were identified with immunohistochemical stain (Fig. 1). These cells were widely distributed, more abundantly in the brain stem, cerebellum, and entorhinal regions. In addition, discrete cortical abnormalities were observed (case 4) with microgyric-like cortical foldings.

In the two remaining pregnancies (cases 6 and 7) no virus was isolated from amniotic fluid culture and no specific IgM antibodies could be found in cord blood. After delivery at term, the newborns were free of infection: no specific IgM antibodies were detected in their serum, and they did not shed virus from urine or saliva within the first 2 weeks of life.

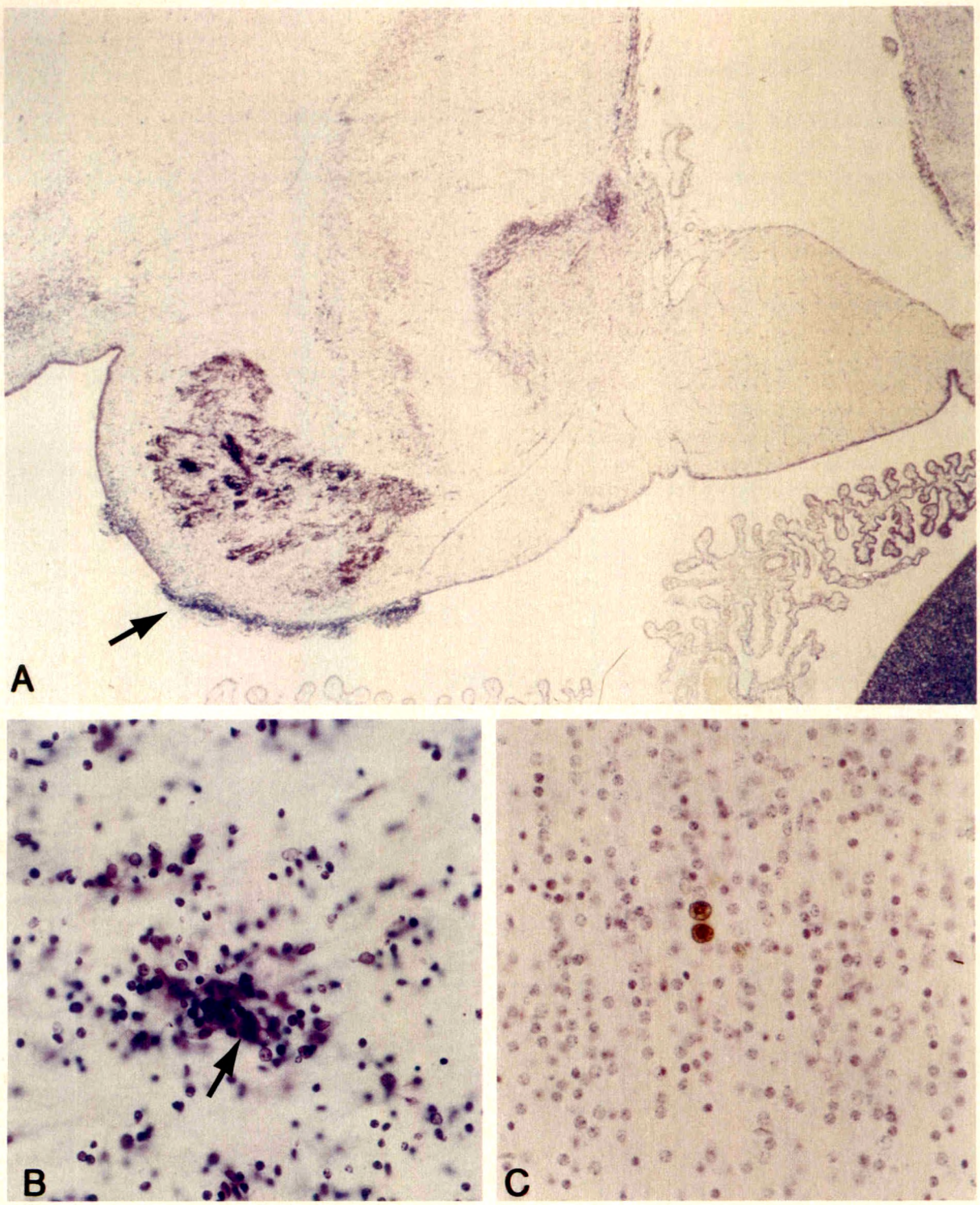


Fig. 1. Brain lesions in cytomegalovirus-infected fetuses. **A**, Perivascular calcified necrotic area and focal ependymal disruptions *(arrows)* by inflammatory cells; coronal section through the entorhinal region. (Hematoxylin-eosin stain; ×12.) **B**, Inflammatory nodule with intracytoplasmic cytomegalic inclusion *(arrows)*, subcortical region. (Hematoxylin-eosin stain; ×150.) **C**, Nuclear cytomegalic inclusions revealed by using an immunoperoxidase staining with anticytomegalovirus monoclonal antibody. Neocortical region. (Hematoxylin-eosin counterstain; ×180.)

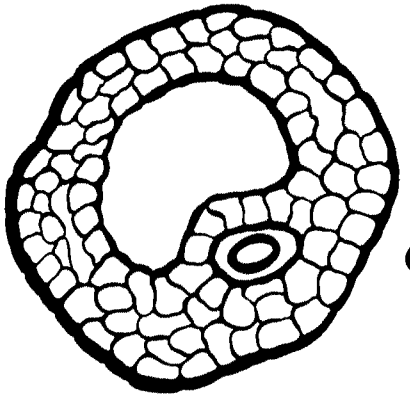
Comment

Detection of specific IgM in fetal blood was considered to be the best marker for prenatal diagnosis of cytomegalovirus infection.[3-7] These antibodies are detected only after 20 weeks of pregnancy.[8] Furthermore, an immature immune system or insufficient test sensitivity could lead to a false-negative result of IgM.[9] We found amniotic fluid culture to be a particularly sensitive indicator of cytomegalovirus transmission. It succeeded in predicting infection in five fetuses, confirmed by viral isolation from various organs and histologic lesions, which included the central nervous system; whether these lesions are responsible for neurologic sequelae requires further studies. Amniotic fluid culture also proved its specificity by showing negative results in the two cases free of infection at birth. We advocate that amniotic fluid culture be used in any case of suspected congenital infection. Two recently published reports dealing with amniocentesis corroborate our observations[10,11]; however, more amniocenteses performed before 22 weeks are needed to assess how early a marker amniotic fluid culture can be.

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Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progastogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. Latter group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myocardial ischemia, occurs with high progestational activity. Consider amount of both steroids in choosing OC. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

Be alert to earliest manifestations of TE disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer with combination or progestogen-only OCs. Studies show no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors and in various subgroups. Decrease in benign breast tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, CIN, erosion, and carcinoma in long-term users. Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunction causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, etc,* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Radiology and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur with OC use. **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES-exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL (of vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,000 births compared to 7.8 for those unexposed ($P<.05$). Female hormone exposure in utero was associated with testicular and ovarian cancer. More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregnant soon after post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, stop OC and apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 study; appeared in 6-12 months' use in another. Bile duct cancer has been reported. **7.** *Carbohydrate and lipid metabolism:* Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides, total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few months and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but increase with longer exposure; in the 5th year it is 2½-3 times the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. **10.** *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. If BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a higher estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. **12.** *Breast feeding:* OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive history observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, unaltered free T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydial and gonorrheal prevalence is increased; do not assume OC protects against chlamydial PID. HIV seropositivity was associated with OC use. *Information for patients.* See patient labeling. *Drug Interactions.* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for premenstrual-like syndrome, cataracts, libido changes, chorea, appatite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hidradenitis suppurativa, hemolytic uremia, malignant hypertension, impaired renal function, acute renal failure, sometimes irreversible, itching, vaginitis, porphyria, PVCs, abnormal EKG, pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use and is available on request.**

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Endothelin-1,2 levels are increased in the amniotic fluid of women with preterm labor and microbial invasion of the amniotic cavity

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The purpose of this study was to determine the effect of gestational age, labor, and microbial invasion of the amniotic cavity on amniotic fluid concentrations of endothelin-1,2. Amniotic fluid was retrieved by amniocentesis from 148 women: patients at term with and without labor, patients with preterm labor with and without intraamniotic infection, and women in the second trimester of pregnancy. Endothelin-1,2 was measured by a sensitive and specific radioimmunoassay. Immunoreactive endothelin-1,2 was detectable in all samples of human amniotic fluid. Advancing gestational age and spontaneous term labor did not result in changes in amniotic fluid concentrations of endothelin-1,2. Women with preterm labor and positive amniotic fluid cultures for microorganisms had higher amniotic fluid concentrations of endothelin-1,2 than did those without microbial invasion of the amniotic cavity ($p < 0.05$). These results support a role for endothelins in the mechanisms responsible for preterm delivery associated with intraamniotic infection. (AM J OBSTET GYNECOL 1992;166:95-9.)

Key words: Endothelins, intraamniotic infection, preterm labor, chorioamnionitis

Endothelins are a family of small proteins that were originally isolated from the culture supernatants of porcine aortic endothelial cells.[1] The first member of this family to be characterized was endothelin-1. Expression of the endothelin-1 gene is regulated by several vasoactive agents, and the peptide is a potent vasoconstrictor. Subsequently, this family of peptides has expanded to include endothelin-1 (porcine/human), endothelin-2, and endothelin-3 (rat endothelin).[2-4] These 21-residue peptides are formed from a preproendothelin (−200 residues) by way of a 38/39-residue intermediate.[1, 2] It is thought that endothelins act as endogenous agonists of dihydropyridine-sensitive calcium channels.[1] Endothelins can induce two types of contractions of the rat uterus,[5] activate phospholipase A_2,[6] and activate phospholipase C.[7, 8] Endothelins have recently been found to be synthesized not only by endothelial cells but also by kidney cells.[9] Moreover, it has been suggested that endothelin-1 is synthesized by amnion.[10, 11]

A role for endothelins in the control of human parturition can be postulated on the basis of their effect on myometrial contractility and on phospholipase A_2 and C. This study was undertaken to determine the effect of labor and microbial invasion of the amniotic cavity on amniotic fluid concentrations of endothelin-1,2.

Material and methods

Patient population and study design. Amniotic fluid was collected from 155 women. Patients were subdivided into four groups: group 1, women in the second trimester of pregnancy (gestational age of 16 to 18 weeks) undergoing amniocentesis for genetic indications (maternal age >35 years) ($n = 22$); group 2, women in the third trimester (gestational age of 38 to 39 weeks) who had amniocentesis for the assessment of fetal lung maturity before elective cesarean section ($n = 25$); group 3, women with spontaneous active labor at term and negative amniotic fluid cultures ($n = 44$). Amniotic fluid was obtained at the time of repeat or primary cesarean section or by amniocentesis in patients who were thought to have preterm labor but who subsequently were delivered of babies weighing >2800 gm and considered term by pediatric examination. Group 4 consisted of women admitted with

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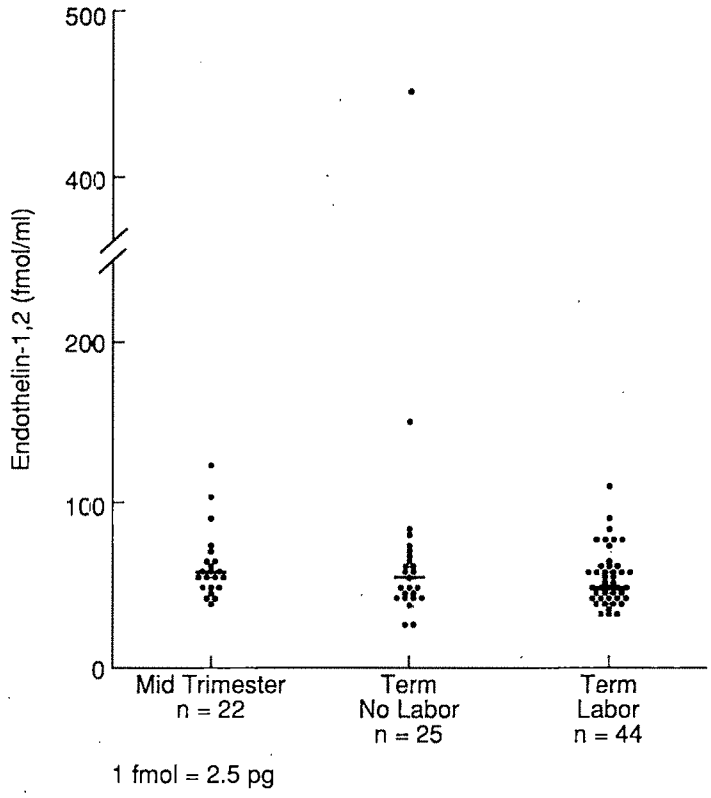


Fig. 1. Amniotic fluid endothelin-1,2 concentrations in women in midtrimester, at term without labor, and at term in active labor. Midtrimester: median 57.5 fmol/ml, range 38 to 125 fmol/ml; term no labor: median 56 fmol/ml, range 26 to 450 fmol/ml; term labor: median 50.5 fmol/ml, range 32 to 110 fmol/ml. No differences among groups ($p > 0.05$).

preterm labor and intact membranes ($n = 57$). Amniocentesis was performed in this group for microbiologic assessment of the amniotic cavity and fetal lung maturity studies. To study the relationship between intraamniotic infection, preterm labor, and amniotic fluid endothelin-1,2 concentrations, a cross-sectional study of patients in group 4 (preterm labor) was constructed. Patients with preterm labor were subdivided into three subgroups according to their response to tocolysis and the results of their amniotic fluid culture: subgroup 4a, women with preterm labor and negative amniotic fluid cultures who were responsive to tocolysis ($n = 21$); subgroup 4b, women with preterm labor and negative amniotic fluid cultures who were unresponsive to tocolysis and were delivered of preterm neonates ($n = 19$); subgroup 4c, women with preterm labor and intraamniotic infection who were delivered of preterm neonates ($n = 17$).

Preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 minutes for at least 60 minutes' duration. Amniocentesis was performed before the initiation of tocolytic therapy with intravenous ritodrine or terbutaline. Failure of tocolysis was diagnosed when cervical dilatation progressed beyond 5 cm and/or delivery occurred. Informed consent was obtained from all patients according to the guidelines of the Yale University Human Investigations Committee.

Microbiologic studies. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after collection and plated within 30 minutes of collection. Fluid was cultured for aerobic and anaerobic bacteria and for *Mycoplasma hominis* and *Ureaplasma urealyticum*, as previously described.[12]

Endothelin-1,2 determinations. Endothelin-1,2 was measured with radioimmunoassay kits from Amersham Corporation (Arlington Heights, Ill.). The assay was performed directly on duplicate 100 μl amniotic fluid samples. We have found that purification of liquor samples before assay has a limited effect on measured values, in marked contrast to its significant impact on plasma measurements. The sensitivity of the assay (2 SD of the zero point binding) is 1.03 fmol. Intraassay and interassay coefficients of variation are 4.1% and 10.4%, respectively.

Statistical analysis. Comparisons of endothelin-1,2 concentrations in amniotic fluid samples from different groups were conducted with a Kruskal-Wallis one-way

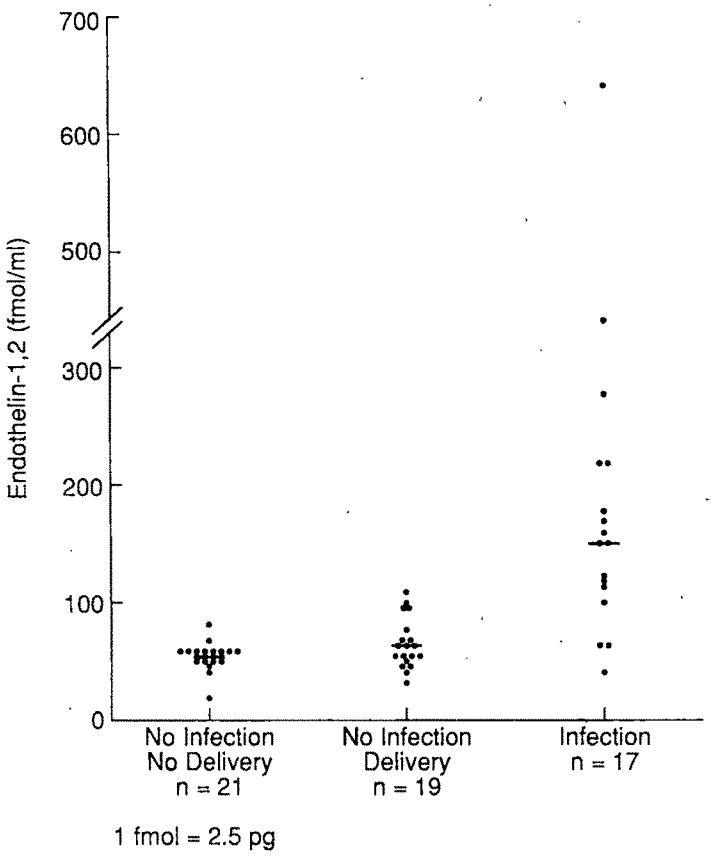


Fig. 2. Amniotic fluid concentrations of endothelin-1,2 in women in preterm labor according to response to tocolysis and amniotic fluid culture results. Subgroup 4a, women with negative amniotic fluid cultures who responded to tocolysis: median 55 fmol/ml, range 17 to 83 fmol/ml; subgroup 4b, women with negative amniotic fluid cultures who did not respond to tocolysis and were delivered of preterm neonates: median 62 fmol/ml, range 31 to 110 fmol/ml; subgroup 4c, women with positive amniotic fluid cultures who were delivered of preterm neonates: median 150 fmol/ml, range 41 to 640 fmol/ml. No difference was found between subgroups 4a and 4b; concentration in subgroup 4c was greater than that in subgroups 4a and 4b ($p < 0.05$).

analysis of variance. The Dunn test was used for post hoc multiple comparisons among groups (True Epistat, Epistat Services, Richardson, Tex.).

Results

Immunoreactive endothelin-1,2 was detected in all amniotic fluid samples from patients in the second and third trimesters of pregnancy (groups 1 and 2, Fig. 1). No changes were observed with gestational age in the amniotic fluid concentrations of endothelin-1,2. Spontaneous labor at term was not associated with an increased concentration of immunoreactive endothelin-1,2 in amniotic fluid ($p > 0.05$) (Fig. 1).

Patients in preterm labor with positive amniotic fluid cultures for microorganisms (group 4c) had significantly greater concentrations of amniotic fluid endothelin-1,2 than did women without intraamniotic infection (subgroups 4a and 4b, Fig. 2) ($p < 0.05$). Failure to respond to tocolysis in women with preterm labor was not associated with higher amniotic fluid concentrations of endothelin-1,2 when compared with those of women in preterm labor who were responsive to tocolysis. Table I displays the clinical and microbiologic information for patients with preterm labor and microbial invasion of the amniotic cavity.

Comment

This study documents the presence of immunoreactive endothelin-1,2 in normal amniotic fluid from women in the second and third trimesters of pregnancy. It is interesting to note that amniotic fluid concentrations of endothelin-1,2 were approximately tenfold higher than those observed in peripheral blood or in the umbilical circulation.[13, 14] No change in the amniotic fluid concentration of endothelin-1,2 was observed with gestational age.

The role of these endothelins is uncertain. Since endothelin can induce smooth muscle contraction, we pos-

Table I. Clinical and microbiologic data of patients with preterm labor and microbial invasion of amniotic cavity

Patient identification No.	*Gestational age (wk)*	*Organism*	*Colony count (cfu/ml)*	*Amniotic fluid endothelin-1 (fmol/ml)*
1	26	*Clostridium* sp.	10^5	
		Capnocytophaga sp.	10^5	115
2	25	Mixed aerobic gram-positive flora	—	220
3	22	*Gardnerella vaginalis*	—	125
4	33	*Fusobacterium* sp	2000	150
5	24	*Gardnerella vaginalis*	$>10^5$	
		Peptostreptococcus sp.	100	440
6	28	*Mycoplasma hominis*	—	
		Fusobacterium sp.	$>10^5$	160
7	29	*Gardnerella vaginalis*	$>10^5$	100
8	28	*Ureaplasma urealyticum*	—	180
9	24	*Ureaplasma urealyticum*	—	
		Streptococcus agalactiae	$>10^5$	63
10	24	*Ureaplasma urealyticum*	—	280
11	28	*Streptococcus viridans*	$>10^5$	63
12	24	*Bacteroides* sp. mixed	$>10^5$	120
13	35	*Candida albicans*	10^5	
		Staphylococcus aureus	$>10^5$	41
14	25	*Streptococcus agalactiae*	$>10^5$	170
15	27	Mixed anaerobes	—	220
16	25	*Ureaplasma urealyticum*	10^5	150
17	33	*Streptococcus viridans*	—	640

tulated that it might play a role in the control of human parturition. Indeed, myometrium is extremely sensitive to the action of endothelin with a contractile effect demonstrated at very low concentrations of the peptide.[15] This action is mediated by an increase in intracellular calcium concentration and phosphorylation of the myosin light chain.[15] Additionally, endothelin-1 can stimulate uterotonic prostaglandin output by myometrium[16] and thus induce contractions indirectly. Our data, however, do not indicate a change in amniotic fluid concentrations of endothelin-1,2 with spontaneous parturition at term.

In contrast, women with preterm labor and microbial invasion of the amniotic cavity have higher amniotic fluid concentrations of endothelin-1,2 than do those with negative amniotic fluid cultures for microorganisms. These results are consistent with a role for endothelin-1,2 in the mechanism of preterm labor associated with intraamniotic infection. Alternatively, the increased availability of endothelin-1,2 in amniotic fluid could be an epiphenomenon associated with parturition in the setting of infection. Further investigation of the intrauterine sources and local regulation of the production of endothelin is required. It is unknown whether bacteria could be a source of endothelin. It has been documented, however, that human amnion produces endothelin[17] and that cytokines such as interleukin-1β can increase endothelin production by amnion.[18] Interleukin-1β is found in the amniotic fluid of women with preterm labor and intraamniotic infection[19, 20] and is produced by human decidua in response to bacterial products.[21] Therefore cytokines produced during the host response to intraamniotic infection may be responsible for the increased availability of endothelin-1,2. Endothelins may play a role in the regulation of myometrial contractility in infection-associated preterm labor. Further studies are required to determine whether amniotic fluid endothelin can cross intact chorioamniotic membranes and reach the myometrium to exert this biologic action.

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Incidence of pelvic inflammatory disease after first-trimester legal abortion in women with bacterial vaginosis after treatment with metronidazole: A double-blind, randomized study

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OBJECTIVE: The purpose of this study was to evaluate the effect of metronidazole treatment on the incidence of postoperative pelvic inflammatory disease after first-trimester abortion in women with bacterial vaginosis.

STUDY DESIGN: A double-blind, randomized, multicenter study was conducted on 231 women undergoing first-trimester legal abortion and fulfilling the criteria for bacterial vaginosis. The women were randomized to either metronidazole 500 mg three times daily for 10 days or placebo. Treatment was started at the outpatient visit the week before the operation.

RESULTS: Among the 174 women who could be evaluated, pelvic inflammatory disease developed in 14 after the abortion. In the treatment group there were three infections (3.8%) compared with 11 (12.2%) in the placebo group ($p < 0.05$).

CONCLUSION: These data suggest that patients with bacterial vaginosis should be treated in conjunction with first-trimester abortion because treatment with metronidazole reduces the postoperative infection rate more than three times. (Am J Obstet Gynecol 1992;166:100-3.)

Key words: Bacterial vaginitis, endometritis, pelvic infection, abortion

The incidence of pelvic inflammatory disease after first-trimester abortion is 4% to 12%.[1-3] Many attempts have been made to identify risk groups. Patients considered to be at risk are women with previous pelvic inflammatory disease, cervical infection with either *Chlamydia trachomatis* or *Neisseria gonorrhoeae,* and women with pregnancy of advanced gestational age.[4, 5] Screening for *C. trachomatis* with subsequent treatment does not, however, reduce the infection rate, indicating to us that other factors might predominate in some patient groups.[6, 7]

Prophylactically administrated antibiotics, i.e., penicillin-ampicillin,[8] metronidazole,[9, 10] tinidazole,[11, 12] and doxycycline,[13, 14] have been tried to reduce the incidence of postabortion pelvic inflammatory disease. With the exception of one study with metronidazole,[10] no risk groups were selected for prophylactic antibiotic treatment. Women with a history of pelvic inflammatory disease received metronidazole, but no reduction in the infection rate could be demonstrated.

Table I. Reasons for exclusion of 57 women from the double-blind study of preoperative metronidazole treatment to patients with bacterial vaginosis undergoing first-trimester elective abortion

C. trachomatis infection	23
Continued pregnancy	7
Other preoperative antibiotic	3
Second-trimester abortion	3
Recurettage	5
Other postoperative antibiotic	6
Prescribed medication not followed	6
Medical records not found	4
TOTAL	57

In a recently published study we showed that women with clue cells in Gram-stained smears may be a group at risk for postabortion pelvic inflammatory disease.[6] The presence of clue cells is one of the accepted signs of bacterial vaginosis and alone is highly discriminative between bacterial vaginosis and normal status.[15] In our study postabortion pelvic inflammatory disease developed in 10.8% of women with clue cells compared with 4.5% of women without clue cells.[6] The aim of this study

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Table II. Rate of postoperative pelvic inflammatory disease after first-trimester abortion in a double-blind study of metronidazole treatment versus placebo in patients with bacterial vaginosis

	Metronidazole			*Placebo*		
		Pelvic inflammatory disease			*Pelvic inflammatory disease*	
Participating clinic	*Patients (No.)*	*No.*	*%*	*Patients (No.)*	*No.*	*%*
Gothenburg	35	1	2.9	44	3	6.8
Skövde	27	1	3.7	22	4	18.2
Gävle	22	1	4.5	24	4	16.7
TOTAL	84	3	3.6	90	11	12.2*

*Metronidazole treatment versus placebo, $p < 0.05$.

was to evaluate whether treatment with metronidazole in women with bacterial vaginosis lowers the rate of postabortion pelvic inflammatory disease after first-trimester abortion.

Material and methods

A double-blind, randomized, placebo-controlled, multicenter study was performed in three different hospitals in Sweden (Sahlgrenska Hospital, Gothenburg; Central Hospital, Skövde; Central Hospital, Gävle). All women >16 years old and without language difficulties visiting our outpatient clinics during the study period for first-trimester legal abortion underwent investigation for the presence of bacterial vaginosis. Bacterial vaginosis was diagnosed if three of the following four criteria were fulfilled: characteristic discharge, pH >4.5, positive amine test, and clue cells in a wet smear.[16] To ascertain uniform diagnosis of bacterial vaginosis at the three hospitals, clue cells were also evaluated on air-dried smears.[17]

All smears were investigated by culture or with Chlamydiazyme (Abbott Scandinavia) for *C. trachomatis*. Culturing for *N. gonorrhoeae* was done in one hospital (Gothenburg). None of the *N. gonorrhoeae* cultures were positive, and because of the low incidence in Sweden culturing was not done at the other two hospitals.[18]

Patients fulfilling the criteria for bacterial vaginosis were invited to take part in the study. After giving verbal consent, the patients were treated with either metronidazole tablets, 500 mg three times daily for 10 days, or placebo tablets. If any complications occurred, the patients were asked to return to the clinic. A vacuum aspiration was done within a week after the outpatient visit and before the twelfth completed week. Preoperative ripening of the cervix was performed in primiparous women with intravaginally administered prostaglandins, Cervagem dilators (Skövde), intracervical laminaria tents (Gävle), or Dilapan dilators (Gothenburg). The medical records of all patients were evaluated at follow-up 1 month later.

Postabortion pelvic inflammatory disease was defined as two or more of the following six criteria fulfilled within 28 days of the operation: (1) abnormal or purulent vaginal discharge after 1 week, (2) abnormal bleeding after the third day, (3) temperature >38.0° C (100.4° F) for >24 hours, (4) palpable adnexal masses, (5) tenderness over the uterus or adnexa on pelvic examination, or (6) erythrocyte sedimentation rate >30 mm.

The metronidazole tablets and identical placebo tablets were supplied by Dumex Ltd., Copenhagen. All data were fed into a computer before the code indicating placebo or metronidazole treatment was broken. The study was approved by the regional ethics committee. The one-tailed z test was used for statistical evaluation. A p value >0.05 was considered nonsignificant.

Results

During the study period 1202 women visited our outpatient clinics for first-trimester abortion. Of these, 231 (19.2%) fulfilled the criteria for bacterial vaginosis and were accepted in the study. The mean age of the study group was 25.2 years. Fifty-seven patients were excluded from the study group, 23 because of *C. trachomatis* infection; all of these received antibiotics before operation. Three women received other antibiotics before operation (two because of *Trichomonas vaginalis* infection). Three women did not undergo the abortion procedure until after 13 weeks of gestation (second-trimester abortion), and seven women continued the pregnancy. Reexeresis was done in five women, six had received postoperative antibiotics, and six did not follow the prescribed medication. The medical records of four patients could not be found (Table I).

The treatment trial thus consisted of 174 women. During the study period 14 cases of postoperative pelvic inflammatory disease occurred. The breakdown of the individual pelvic inflammatory disease criteria were as follows: 10 women had tenderness over the uterus or adnexa on pelvic examination in combination with fe-

ver and the other four women had tenderness over the uterus or adnexa in combination with abnormal bleeding ($n = 2$) or discharge ($n = 2$) and one of them had palpable adnexal masses. Three (3.6%) of the 84 patients in the metronidazole group and 11 (12.2%) of the 90 patients in the placebo group ($p < 0.05$; Table II) had postoperative pelvic inflammatory disease. Treatment of bacterial vaginosis lowers the incidence of post abortion pelvic inflammatory disease by 8.7% (95% confidence limits 0.9% to 16.6%); thus treatment lowers the infection more than 3 times.

The median time from abortion to infection were 5.5 days. The time to infection did not exceed 16 days, and the women treated with metronidazole have the infections in the first 4 days. The infection rates in the three hospitals are listed in Table II. Statistically there were no significant differences.

The investigation of air-dried smears according to the described method[17] was done after the completion of the study. Of the 174 women in the study, eight smears could not be examined because of either interfering blood or little material for correct diagnosis. Thus it was possible to examine 166 air-dried smears. Of these, 158 contained clue cells (95.2%). None of the eight patients without clue cells in air-dried smears had a postoperative infection.

Comment

In an attempt to reduce the risk for postoperative infection associated with first-trimester elective abortion, several studies have been performed to define criteria for prophylactic treatment with antibiotics. The only risk groups identified so far are women with a positive culture for *N. gonorrhoeae* or *C. trachomatis*.[5, 14, 19] Screening is therefore often performed for *C. trachomatis* infection among (all or) selected groups of women undergoing first-trimester abortion.[2, 19, 20]

Bacterial vaginosis signifies a disturbed or abnormal vaginal flora, with suppression of lactobacilli and an increase in the number of other bacteria such as *Mycoplasma hominis*, bacteroides, anaerobes, and *Gardnerella vaginalis*.[21] It is conceivable that during abortion these microorganisms can ascend into the endometrial cavity[7] and in some cases cause infection. Many postabortion infections occur within 1 week after the operation, and it has been questioned whether these early infections are caused by *C. trachomatis*.[2, 11] Early infections that mostly occur in the endometrial cavity may not be as hazardous to future fertility as salpingitis that develops after 2 to 4 weeks.[22] Such salpingitis is probably more often caused by *C. trachomatis*.[2, 11] The early infections, however, will still cause a great deal of discomfort and morbidity for the patients, with an increase in hospitalization. Studies published on the role of *C. trachomatis* in postabortion pelvic inflammatory disease have not divided the infections into early and late.[1-4, 14, 19, 20] Metronidazole treatment is effective only against anaerobic bacteria associated with bacterial vaginosis and not against *C. trachomatis;* therefore the reduction in infection rate in our study is most probably not due to undetected *C. trachomatis* infections, although *C. trachomatis* is associated with bacterial vaginosis. The incidence of *C. trachomatis* was 10.0% in our study group but only 2.6% among the other women not having bacterial vaginosis. This association between bacterial vaginosis and *C. trachomatis*–infected patients attending sexually transmitted disease clinics has been shown previously.[23, 24] The results of previous studies[1, 14, 20] demonstrating a reduction in postabortion pelvic inflammatory disease if *C. trachomatis*–positive women are treated with tetracyclines could thus be because tetracyclines are also effective against bacterial vaginosis.

It is also known that the experience of the surgeon is of importance, but since all colleagues taking part in the study at the three departments were either attending surgeons or resident surgeons under training and were supervised by attending surgeons, this should not have interfered with our results.

Our results indicate that patients with bacterial vaginosis should be treated in conjunction with first-trimester elective abortion to reduce the risk of postabortion infection. These results are partly supported by other studies that have found connections between preterm birth,[25] postcesarean endometritis, and bacterial vaginosis diagnosed by vaginal Gram-stained smears.[26] Recently a connection has been shown between bacterial vaginosis and postoperative infection after abdominal hysterectomy,[27] a connection that we have also found.[28]

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Depressive symptoms in women in the six months after miscarriage

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This study, the first systematic investigation of the psychiatric impact of miscarriage, tests whether miscarriage markedly increases depressive symptoms in the 6 months after loss. We interviewed 382 miscarrying women entering the study at 2 weeks, 6 weeks, or 6 months after loss and, for comparison, 283 pregnant women and 318 community women not recently pregnant. Among women interviewed 2 weeks after miscarriage the proportion highly symptomatic on the Center for Epidemiologic Studies–Depression scale was 3.4 t mes that of pregnant women (95% confidence limits 2.0 and 5.0) and 4.3 times that of community women (95% confidence limits 3.0 and 5.8). Among women first interviewed 6 weeks and 6 months after miscarriage the proportion highly symptomatic was three times that of the community women. Women reinterviewed at 6 weeks and 6 months did not have elevated symptom levels, a result attributed to the unintended therapeutic and test effects of study interviews. Interviews were fully structured, readily administered by telephone by nonmedical personnel. The possibility that such interviews afford miscarrying women substantial psychologic benefits merits future investigation. (AM J OBSTET GYNECOL 1992;166:104-9.)

Key words: Spontaneous abortion, depression, grief counseling, miscarriage, pregnancy

Twelve percent to 15% of clinically recognized pregnancies end in spontaneous abortion.[1] For many women miscarriage represents death of a future child, dislocates reproductive plans, and may engender doubts about procreative competence. Nonetheless society lacks established rituals for mourning such loss.[2, 3]

In spite of repeated calls for studies of the psychiatric sequelae of reproductive loss[2, 4-6] relevant investigations are rare, and methodologic shortcomings cloud their findings. Only three investigations focused solely on miscarriage[7-9]; an additional six included miscarriage (loss usually at >20 weeks' completed gestation) in investigations of perinatal death.[10-15] These studies lacked a comparison group unexposed to recent reproductive loss, thereby precluding estimation of its effect on depressive symptoms. Wide variation in the timing of subject assessment ranging within a given study from a few weeks to months or even years after loss,[11, 12, 15] small samples, and absence of data on response rates[11, 13] further complicate evaluation and interpretation of research findings.

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Material and methods

Study design. The study comprised three cohorts: miscarrying women, pregnant women, and women in the community not pregnant in the preceding year. Levels of depressive symptoms in the miscarrying women were compared with those in these two other groups.

Miscarriage cohort. Miscarriage was defined as the involuntary termination of a nonviable, intrauterine pregnancy before 28 completed weeks of gestation with the conceptus dead on expulsion. The miscarriage cohort was derived from the cases of an antecedent hospital-based case-control study of risk factors for miscarriage[16] in which 77% of all women attending a New York City medical center for miscarriages from late 1984 to 1986 were interviewed. This antecedent case-control study interviewed approximately 75% of these cases within 2 weeks of miscarriage, 95% within 8 weeks, and the remainder by 6 months.

Pregnant cohort. The pregnant cohort was derived from the controls of the case-control study and consisted of women registered for prenatal care before 22 weeks' gestation selected to be similar to the miscarrying women in age and whether they were receiving medical

Table I. Selected sociodemographic characteristics of women in miscarriage, pregnant, and community cohorts

Sociodemographic Characteristic	*Cohort*		
	Miscarriage (n = 382)	*Pregnant (n = 283)*	*Community (n = 318)*
Age (yr, mean ± SD)	29.2 ± 6.2	28.9 ± 5.7	30.0 ± 6.4
Ethnicity (%)			
White	32.2	36.4	36.6
Black	20.7	15.5	19.6
Hispanic	39.8	44.9	38.2
Other	7.3	3.2	5.7
Interviewed in Spanish (%)	32.7	25.8	26.4
Education (%)			
Less than high school	28.6	24.7	20.3
High school graduate	24.1	20.9	24.5
Some college	22.8	25.8	29.8
College graduate plus	24.6	28.6	25.5
Income (%)*			
<$9,000	37.1	39.6	23.9
≥$40,000	24.7	26.9	22.2
Current marital status (%)*			
Single	26.7	22.6	46.5
Married	60.2	60.8	35.2
Other	13.1	16.7	18.3

*$p < 0.01$ for overall differences across cohorts. For categorical variables and age, differences among groups were evaluated by an overall χ^2 test and one-way analysis of variance, respectively.

care privately or in a public, clinic setting. Eighty-five percent of identified controls were interviewed for the case-control study. Of identified cases and controls, participants and nonparticipants in the case-control study were similarly distributed on sociodemographic and reproductive characteristics.[16]

At the conclusion of the case-control interview all participants who were 18 years of age or older, English or Spanish speaking, and accessible by telephone were invited to participate in our study. For pregnant women we also required that the case-control interview occur before 28 weeks' completed gestation.

We aimed to assess miscarrying women at three time points: the second week after miscarriage (2 weeks), weeks 6 through 8 (6 weeks), and weeks 26 through 35 (6 months). Overall, 73% (n = 382) of eligible miscarrying women were evaluated at least once; 232 were first interviewed at 2 weeks, 114 at 6 weeks, and 36 at 6 months. This staggered recruitment arises largely from the timing of the antecedent case-control study interview and logistic difficulties encountered by the current study in scheduling women for assessments within narrow, fixed time intervals after loss.

Seventy-two percent (n = 283) of eligible pregnant women were interviewed on a single occasion

Community cohort. Recruits for the community cohort were located by randomly dialing telephone numbers based on the telephone area codes and exchanges of the interviewed miscarrying women. The eligibility of household residents was established for 92% of working telephone numbers. Community women were frequency matched to the women in the miscarriage cohort on language of interview, age (in 5-year intervals), education (less than high school, high school graduate, college graduate, postgraduate training), and season of interview. Of (known) eligible community women, 82% (n = 318) were interviewed on a single occasion.

Comparability of interviewed and uninterviewed subjects. Within each cohort interviewed and uninterviewed women were similar in age, ethnicity, education, language, and season of interview. Interviewed and uninterviewed miscarrying and pregnant women also did not differ on private-public status, marital status, parity, or prior reproductive loss. For miscarrying women mean length of gestation at loss was slightly greater (8 to 9 days) for interviewed women compared with those not interviewed. Mean scores on a measure of depressed mood[17] did not differ between interviewed and uninterviewed women.

Study measures

The presence and duration of depressive symptoms in the week before the interview were evaluated with the 20-item Center for Epidemiologic Studies-Depression (CES-D) scale.[18] This scale measures affective, cognitive, and somatic components of depression. Each item is scored from 0 to 3 depending on the frequency of the symptom and is summed to produce an overall index. The CES-D correlates well with other self-report

Table II. Selected reproductive history characteristics of women in miscarriage, pregnant, and community cohorts

Characteristic	Cohort		
	Miscarriage (n = 382)	*Pregnant (n = 283)*	*Community (n = 318)*
Private payment of hospital bill (%)	34.0	39.2	—
Reproductive‡			
Nulliparous (%)	36.6	34.6	50.5
Living children (%)			
0	38.0	35.3	50.5
1	29.9	37.8	15.7
2	18.3	18.4	19.7
3+	13.8	8.5	14.1
Prior reproductive loss (%)*			
0	66.0	71.0	82.5
1	22.3	20.8	12.9
2+	11.7	8.2	4.6
Weeks' gestation (%)†			
<8	10.5	—	—
8-11	38.4	0.7	—
12-15	24.3	5.3	—
16-19	15.2	16.6	—
20-23	8.4	33.9	—
24-27	3.2	36.8	—
28+	—	6.7	—

*Includes spontaneous abortions (89%), fetal deaths (5%), ectopic pregnancies (3%), and neonatal deaths (3%).

†For the miscarriage cohort, the interval refers to time from last menstrual period to date of expulsion of conceptus; for the pregnant cohort, the interval refers to time from last menstrual period to miscarriage study interview.

‡p Value < 0.01 for overall differences across cohorts. Differences among groups were evaluated by one-way analysis of variance.

Table III. Comparison of CES-D means within miscarriage cohort across time points and by interview status

Time point	*Women first interviewed at 2 wk*		*Women first interviewed at 6 wk or 6 mo*		*Adjusted difference in means (95% CL)**	
	No.	*Observed CES-D (mean ± SEM)*	*No.*	*Observed CES-D (mean ± SEM)*	*Across time points: Women first interviewed at 2 wk vs women first interviewed later*	*Within time points: Reinterviewed women vs first interviewed women*
2 wk	232	23.9 ± 0.8	—	—	—	—
6 wk	196	13.7 ± 0.8	114	18.8 ± 1.2	4.0 (1.1, 6.8)	6.1 (3.4, 8.8)
6 mo	266	13.8 ± 0.7	36	20.6 ± 2.3	3.3 (−1.1, 7.7)	6.3 (2.1, 10.5)

CL, Confidence limits.

*Means are adjusted by ordinary least-squares multiple regression analysis.

measures of depressive symptoms, distinguishes clinically depressed patients from the general population, and has been used previously with samples of pregnant women.[19] A CES-D mean of 24.4 has been reported for heterogeneous samples of psychiatric inpatients.[18] The CES-D was administered by telephone[20] and preceded an extensive interview covering the woman's sociodemographic characteristics, social supports, attitude toward the pregnancy, and reproductive history, including history of the current pregnancy and miscarriage. Interviewers were not required to have clinical training or experience.

Sample characteristics. The three cohorts were similarly distributed on the majority of sociodemographic variables (Table I). Overall mean age was 30 years; 40% were Hispanic; a quarter had not graduated from high school; approximately 30% had incomes of <$10,000. As expected, the two hospital-based cohorts contained fewer single and childless women and had more reproductive loss (Table II). Half of index miscarriages occurred at <12 weeks' gestation; the majority of pregnant women were interviewed after the first trimester.

Analytic strategy. A first set of analyses tested differences in symptom levels within the miscarriage co-

Table IV. Comparison of proportions of subjects scoring ≥30 on the CES-D: Miscarriage cohort at three time points after loss (stratified by interview status) with pregnant and community cohorts at one time point

Cohort (n)	*Observed percent scoring ≥30 (mean ± SEM)*	*Adjusted ratio of proportions**	
		Miscarriage to pregnant cohorts (95% CL)	*Miscarriage to community cohorts (95% CL)*
Pregnant (283)	12.4 ± 2.0	—	—
Community (318)	10.1 ± 1.7	—	—
Miscarriage, 2 wk			
First interview (232)	36.2 ± 3.2	3.4 (2.0, 5.0)‡	4.3 (3.0, 5.8)
Miscarriage, 6 wk			
First interview (114)	20.2 ± 3.8	—	2.6 (1.5, 4.4)
Reinterview (196)	11.7 ± 2.3	—	1.4 (0.8, 2.4)
Miscarriage, 6 mo			
First interview (36)	30.6 ± 7.7	—	3.0 (1.2, 5.9)
Reinterview (266)	12.8 ± 2.0	—	1.5 (0.9, 2.5)

CL, Confidence limits.

*Proportions are adjusted with maximum likelihood logistic regression analysis.

†Odds ratios (95% CL) for miscarriage-community comparisons in descending-column order: 6.2 (3.6, 10.5); 3.1 (1.5, 6.2); 1.4 (0.7, 2.7); 3.6 (1.3, 10.2); 1.6 (0.9, 2.9).

‡Odds ratio (95% CL) for this comparison is 4.9 (2.4, 10.0).

hort across time points. A second set tested differences in symptom levels between the miscarriage cohort and the two comparison groups. At 2 weeks after loss the miscarriage cohort was compared with both the pregnant and community cohorts. Thereafter, because the pregnant cohort offered an estimate of symptom levels among miscarrying women immediately before loss, it lacked pertinence beyond the 2-week mark. Depressive symptom levels did not vary with length of gestation at loss. Consequently early and late miscarriages were combined in these analyses.

CES-D symptom levels were treated as continuous and categorical variables. The categorical approach tested whether findings based on the entire range of CES-D scores hold for extreme symptom levels. Subjects were dichotomized so that women receiving CES-D scores of ≥30, that is, those who reported at least half of the 20 symptoms in the CES-D for at least 1 week, were categorized as highly symptomatic. Approximately two thirds of these individuals would be expected to meet diagnostic criteria for major depressive disorder.[21] Analyses were conducted with ordinary least-squares multiple regression and maximum likelihood logistic regression to permit adjustment for potentially confounding variables. Comparisons of miscarrying with community women controlled for maternal age, ethnicity, marital and socioeconomic status, number of living children, and prior reproductive loss. Comparisons with pregnant women, as well as those within the miscarriage cohort, also controlled for private-public status, length of gestation (at time of miscarriage at interview), and pregnancy attitude. Results from logistic regression analyses are presented in terms of ratios of proportions scoring ≥30 and odds ratios, the latter reserved for notes to the relevant tables. Statistical significance was assessed at $p < 0.05$.

Results

Comparisons within the miscarriage cohort. In the miscarriage cohort at 2 weeks after loss the observed symptom mean is 23.9 (Table III), with 36.2% of the subjects being highly symptomatic (Table IV). At 6 weeks and 6 months after loss, depressive symptom levels are lower than at 2 weeks but substantially so only among reinterviewed women. Between miscarrying women first interviewed at 2 weeks and those first interviewed at later time points, adjusted differences in means at 6 weeks are 4.0 points and at 6 months 3.3 (Table III). Between women first interviewed and those reinterviewed at the same times, the differences are considerable. At 6 weeks their adjusted symptom means differ by 6.1 points and at 6 months by 6.3 points. The same pattern of results holds in analyses based on proportions highly symptomatic.

Comparisons between study cohorts. In the miscarriage cohort at 2 weeks after loss the adjusted symptom mean exceeds those in the pregnant and community cohorts by 7.5 to 9.1 points, respectively (Table V). Among miscarrying women receiving their first interviews at 6 weeks and 6 months symptom means were still significantly greater than that in the community cohort (5.2 and 5.9, respectively). Similarly, at 2 weeks after loss the proportion of highly symptomatic subjects in the miscarriage cohort is 3.4 times that in the pregnant cohort and 4.3 times that in the community cohort (Table IV). Among miscarrying women first inter-

Table V. Comparison of CES-D means: Miscarriage cohort at three time points after loss (stratified by interview status) with pregnant and community cohorts at one time point

Cohort (n)	*Observed CES-D (mean ± SEM)*	*Adjusted difference in means**	
		Between miscarriage and pregnant cohorts (95% CL)	*Between miscarriage and community cohorts (95% CL)*
Pregnant (283)	15.2 ± 0.7	—	—
Community (318)	14.6 ± 0.8	—	—
Miscarriage, 2 wk			
First interview (232)	23.9 ± 0.8	7.5 (4.5, 10.5)	9.1 (6.9, 11.2)
Miscarriage, 6 wk			
First interview (114)	18.8 ± 1.2	—	5.2 (2.6, 7.8)
Reinterview (196)	13.7 ± 0.8	—	−1.0 (−3.1, 1.1)
Miscarriage, 6 mo			
First interview (36)	20.6 ± 2.3	—	5.9 (2.0, 9.8)
Reinterview (266)	13.8 ± 0.7	—	−0.6 (−2.6, 1.5)

CL, Confidence limits.

*Means are adjusted by ordinary least-squares multiple regression analysis.

viewed at 6 weeks and 6 months, the proportions that are highly symptomatic are 2.6 and 3 times that in the community cohort, respectively. Among women reinterviewed at 6 weeks or 6 months, symptom levels do not differ from those in the community cohort.

The pattern of findings described persisted after controlling for the contribution of somatic symptoms to the overall CES-D score. Hence symptom levels reported by study subjects do not reflect simply somatic complaints arising from the physical sequelae of the miscarriage (or of the pregnancy). In addition, while one third of miscarrying women were hospitalized overnight and another third for ≥2 nights, hospital stay was not associated with symptom levels. Women who had dilatation and curettage procedures (approximately 85%) and those who did not also did not differ on level of depressive symptoms. Finally, women with early and late miscarriages reported similarly elevated symptom levels. For example, 34.1% of women with first-trimester miscarriages (12 completed weeks' gestation) were highly symptomatic, as were 39.8% of women with later loss ($p < 0.40$).

Comment

Among miscarrying women depressive symptoms are elevated fourfold over those in the community women in the second week after miscarriage and 3.4-fold over those in pregnant women. These results document for the first time the magnitude of the early effect of miscarriage on maternal psychologic status. Levels remain high at 6 weeks and at 6 months among women first interviewed at these time points but not among the reinterviewed. This difference in symptom levels between women first interviewed at 6 weeks and 6 months and those being reinterviewed suggests a strong therapeutic effect of study interviews. However, alternative explanations for this finding need to be considered first.

Selection bias in the form of selective departure or selective deferment of participation by more depressed study subjects is unlikely to contribute substantially to these results. First, as to selective departure, women interviewed at 2 weeks or at 6 weeks after loss who thereafter left the study did not differ on symptom levels from women who remained. Second, as for selective deferment, adjusted scores on the depressed mood scale administered at the time of the case-control interview did not differ among women first interviewed at 2 weeks, 6 weeks, and 6 months. Furthermore, women first interviewed at 2 weeks and 6 weeks had nearly identical symptom levels at 6 months (13.6 and 14.1, respectively). Similarly, in a large community survey of depressive symptoms, subjects interviewed in the calendar week targeted for study assessment and those deferring to a later time point did not differ on CES-D symptom levels.[22]

The lower scores of reinterviewed women are more easily explained as a consequence of unintended benefits of study participation, together with a possible test effect arising from scale repetition. Our initial interview afforded each subject an opportunity to discuss her pregnancy, the circumstances of the miscarriage, and its psychologic aftermath. In this respect it inadvertently resembled grief counseling.[7, 23] The efficacy of such counseling, often propounded, has been rigorously demonstrated in at least one randomized, controlled trial.[23] After a perinatal death women in the treatment group received a modal number of two counseling sessions. At 6 months after loss, the rate of psychiatric disorder in the treated group was one quarter that in the untreated group (12.5% and 52.6%, re-

spectively). The first interview with miscarrying women in our study lasted from 1.5 to 3 hours and was generally given in two parts on separate days.

Our estimates of a twofold to threefold elevation in depressive symptoms at 6 weeks and 6 months after loss were based on miscarrying women receiving their first interview; these estimates accord well with the previous controlled investigation of reproductive loss. Clarke and Williams[24] compared depressive symptoms in women experiencing perinatal death with depressive symptoms in women having live births. (The study protocol did not involve extended personal interviews with subjects.) At 6 weeks and 6 months thereafter the proportions of depressed women were 2.9 and 2.4 times those of women with live births, respectively. The results from the study of grief counseling cited above[23] also argue for at least a fourfold elevation in psychiatric symptoms at 6 months after loss in the absence of intervention. Given the preceding evidence based on both our own data and those of these other reports, we take symptom levels at first interview at each time to represent a better estimate of the true magnitude of the effect of miscarriage than levels at reinterview.

Previous authors have suggested that the "conspiracy of silence"[2] surrounding reproductive loss or the part of hospital staff, family, and friends exacerbates depressive reactions. It would not be at all surprising therefore if our interview, with its direct focus on the miscarriage, proved to be psychotherapeutic. Although we had anticipated some drop in the subjects' reported symptoms as a result of study interviews, the magnitude of the observed decline was unexpected. The efficacy of this proposed method of intervention requires rigorous a priori testing. Nonetheless, on the basis of these initial findings, structured telephone interviews administered by lay personnel should have strong clinical and budgetary appeal for medical institutions unable to offer formal bereavement counseling. These results should also prompt obstetricians to meet with a miscarrying patient soon after loss simply to afford her a potentially therapeutic opportunity to review the circumstances of the pregnancy and the miscarriage.

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Alloimmune thrombocytopenia may be associated with systemic disease

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Adverse outcome was encountered in a case of neonatal alloimmune thrombocytopenia after in utero platelet transfusion. This may have resulted from generalized systemic vascular endothelial damage, because several cell types, including umbilical endothelial cells, have been shown to contain surface molecules similar to the receptor containing the PI^{A1} antigen. (Am J Obstet Gynecol 1992;166:110-1.)

Key words: Neonatal alloimmune thrombocytopenia, in utero platelet transfusion

Neonatal alloimmune thrombocytopenia resulting from material antiplatelet antibodies against the PI^{A1} surface antigen on fetal platelets may cause fetal intracranial hemorrhage. Suggested therapies have included fetal in utero platelet transfusions and maternal intravenous γ-globulin infusion. Here we present a case from our institution in which platelet transfusion and immunoglobulin therapy were associated with significant fetal acidosis and subsequent infant death.

Case report

Our patient was seen during her seventh pregnancy (three miscarriages before 12 weeks, one ectopic pregnancy, and two elective abortions). The diagnosis of alloimmune thrombocytopenia was made after her delivery of a term female infant with multiple petechiae and severe thrombocytopenia with a normal maternal platelet count. That infant had neonatal seizures after platelet transfusions. She subsequently had slow gross and fine motor development and an attention deficit disorder. No head ultrasonographic examination was done at birth, but computed tomographic scans obtained at 6 years of age excluded structural lesions. During the current pregnancy, the patient was offered enrollment in the protocol of Bussel et al.[1] for exogenous immunoglobulin therapy. However, the patient did not wish to undergo multiple cordocenteses, and empiric weekly immunoglobulin injections were begun at 21 weeks' gestation. At 35 weeks the patient underwent amniocentesis and cordocentesis for determination of fetal lung maturity, need for further therapy, and route of delivery. Cordocentesis was performed, and an in utero platelet transfusion of 10 ml irradiated maternal platelets (collected by pheresis the day before the procedure) was administered while fetal platelet count results were awaited. This was terminated after approximately 5 minutes when the count was reported as 90,000/mm³. Shortly after, a fetal bradycardia was noted and, although it eventually resolved (within approximately 20 minutes), there were additional variable decelerations. Three hours later a cesarean section was performed, and a female infant was delivered. External fetal monitoring within 5 minutes of delivery showed a fetal heart rate >110 beats/min. The initial platelet count of the baby was 120,000/mm³, which was what we had anticipated on the basis of our calculations from the known amount of platelets transfused. The amniotic fluid was clear, and examination of cord and placenta failed to reveal a thrombosis or abruptio placentae. Computed tomographic and magnetic resonance imaging scans of the infant's brain on days 3 and 5 after birth did not show any focal intracranial lesions. The infant was neurologically impaired and eventually died of bronchopneumonia at 6 months of age. Autopsy examination revealed gliosis and multifocal neuronal loss consistent with perinatal hypoxia-ischemia.

Comment

The cause of this adverse outcome is uncertain because the procedure was performed without obvious technical complications. Interestingly, Giltay et al.[2] have reported that fibroblast cells of the umbilical endothelium, umbilical artery smooth muscle, and human foreskin express a surface molecule that is similar, if not identical, to the endothelial fibronectin receptor that contains a β-subunit carrying the PI^{A1} antigen. This suggests that other cells, aside from platelets, may be involved in the pathophysiologic characteristics of alloimmune thrombocytopenia and that systemic vascular damage could result in this disease. Such damage could cause early in utero intracranial microhemorrhages. Similar damage in the umbilical vessels could predispose to vasospasms which, in turn, could aggravate the underlying neurologic impairment. Alternatively, infusing platelets and associated products could

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aggravate impaired vascular endothelium, causing vasospasms. It is interesting to note that the first child had seizures after platelet transfusion and now has a learning disability. Of note, an ultrasonographic examination of the current fetus had shown a −1.9 SD in the head circumference at 29 weeks, but follow-up scan showed a less significant lag. Although unproved, it is also possible that some earlier in utero event, i.e., hemorrhage, had taken place before therapy was initiated.

There is still much unknown regarding the pathophysiology of this disease and the effects of therapy, such as exogenous immunoglobulins and platelet transfusions, on the long-term outcome of these infants. Platelet transfusions would be the most reliable way to elevate the platelet count. However, they need to be performed with caution. Immunoglobulin treatment may be a better alternative, as our patient's platelet count was 90,000/mm^3 with weekly intravenous doses of immune globulin. Although studies of long-term immune globulin therapy in patients with immunodeficiency syndrome have failed to reveal any significant risks to date, the long-term consequences of maternal-fetal intravenous immune globulin therapy have yet to be defined.

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Spontaneous abortion and subsequent adverse birth outcomes

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OBJECTIVE: Our purpose was to evaluate the association between spontaneous abortion and subsequent adverse birth outcomes.
STUDY DESIGN: Washington State birth certificate records for 1984 to 1987 were used to examine the association between spontaneous abortion and adverse outcomes in the subsequent live birth. Adverse birth outcomes were examined for women with one spontaneous abortion before the index pregnancy (n = 2146) and for women with three or more prior spontaneous abortions and no other prior pregnancies (n = 638); compared with women with no prior spontaneous abortions (n = 3099). Logistic regression was used to estimate the relative risk associated with prior spontaneous abortion of each adverse outcome.
RESULTS: Women with three or more prior spontaneous abortions were at higher risk for delivery at <37 weeks' gestation (relative risk 1.5, 95% confidence interval 1.1 to 2.1), placenta previa (relative risk 6.0, 95% confidence interval 1.6 to 22.2), having membranes ruptured >24 hours (relative risk 1.8, 95% confidence interval 1.2 to 2.9), breech presentation (relative risk 2.4, 95% confidence interval 1.6 to 3.6), and having an infant with a congenital malformation (relative risk 1.8, 95% confidence interval 1.1 to 3.0).
CONCLUSION: These findings suggest that common causes may underlie recurrent spontaneous abortion and certain adverse birth outcomes. They may also help guide clinical management of pregnancies in women with a history of recurrent spontaneous abortions. (Am J Obstet Gynecol 1992;166:111-6.)

Key words: Spontaneous abortion, preterm delivery, congenital malformation

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Spontaneous abortion before the 20th week of gestational age is reported to occur in 15% to 20% of recognized pregnancies.[1, 2] Approximately 1% of women experience three or more spontaneous abortions before the first live birth.[1] In spite of the frequency of this problem, the subsequent birth outcomes of women with prior spontaneous abortions have not been extensively studied. Previous studies have suggested

that women with a history of spontaneous abortion are at increased risk for preterm delivery,[3-5] placenta previa,[6] and having an infant with intrauterine growth retardation (IUGR)[7, 8] or congenital malformation.[9, 10] These studies have been limited by small sample sizes[5-9] and a lack of consideration of possible confounding variables.[3-10]

The current study sought to examine the incidence of adverse birth outcomes among women with one prior spontaneous abortion and women with three or more prior spontaneous abortions, compared with the incidence among women with no prior spontaneous abortions. We used a large data base that includes information on maternal age, parity, gravidity, smoking status during pregnancy, race, and prenatal care.

Material and methods

Data were taken from Washington State birth certificate records for the years 1984 through 1987. Obstetric ward clerks are generally responsible for abstracting information from the medical chart into the birth certificate form. Handbooks describing procedures for birth certificate completion are provided by the State of Washington. Additional instruction is provided by each hospital. A state field representative makes periodic visits to review completion procedures. Most adverse birth outcomes are represented by a checklist format. The completeness of reporting birth complications on the Washington State birth certificate was the subject of a recent study.[11] With the checklist format, reporting was 80% or better for most birth complications (e.g., preeclampsia, breech presentation, and cesarean section). Congenital malformations were reported less frequently, as judged by hospital discharge diagnoses. Reporting rates for congenital malformations ranged from 32% for polydactyly to 57% for cleft lip and palate. The birth certificate defines spontaneous abortion as fetal loss occurring before 20 weeks' gestation; first- and second-trimester spontaneous abortions are not differentiated.

Two "exposure" groups were constructed. The group of women with one prior spontaneous abortion consisted of all women having a live singleton birth in 1987 who had one prior spontaneous abortion and no other prior pregnancies. A second group of women with recurrent spontaneous abortions consisted of all women having a live singleton birth in 1984 to 1987, who had three or more prior spontaenous abortions and no other prior pregnancies. Births ending in fetal death were not included because of their small numbers (<0.7% of all pregnancies >20 weeks' gestation).

The unexposed comparison group consisted of 3099 randomly selected women having a live singleton birth in 1987 with no pregnancies prior to the index pregnancy. Use of this primigravid group thus controlled for the potential confounding effects of parity.

The adverse birth outcomes of interest were: low birth weight (<2500 gm), IUGR, preterm birth (<37 weeks' gestational age, calculated from the first day of the last menstrual period), congenital malformations, prolonged rupture of membranes (>24 hours), breech presentation, low 1-minute (<6) and 5-minute (<7) Apgar scores, abruptio placentae, placenta previa, and preeclampsia. The definition of a low Apgar score was chosen to allow comparison of the results with those of a previous study.[10] Intrauterine growth retardation was defined as birth weight <10th percentile for gestational age as determined by last menstrual period. The 10th percentile of birth weight by menstrual weeks' gestation was taken from a widely cited nomogram and table of birth weights.[12]

Maximum likelihood logistic regression[13] was used to estimate the relative risk associated with prior spontaneous abortion for each adverse birth outcome while controlling for confounding variables.

Results

The characteristics of women with one prior spontaneous abortion, three or more prior spontaneous abortions, and the primagravid comparison group are shown in Table I. Women with three or more spontaneous abortions were older and more likely to smoke, compared with the primigravid group. Women with one prior spontaneous abortion were intermediate for these characteristics. The differences in the proportion of smokers among women with one prior spontaneous abortion and women with three or more prior spontaneous abortions, compared with the proportion among women with no prior spontaneous abortions, were highly significant ($p < 0.001$) after controlling for age. The differences by maternal race, marital status, urban residence (living in an incorporated area), and prenatal care were relatively small.

Information was missing on <3% of the outcome variables examined, except for gestational age, congenital malformations, and preeclampsia. Gestational age was missing for 7% of the group with one prior spontaneous abortion, 9% of the group with the three or more prior spontaneous abortions, and 9% of the primigravid group. The totals missing for those with malformations were 14%, 13%, and 16%, respectively. All three groups had 10% of the preeclampsia data missing.

The frequencies of adverse outcomes by group are presented in Table II. Differences were found for low birth weight, IUGR, preterm delivery, congenital malformation, placenta previa, prolonged rupture of membranes, breech presentation, 1-minute and 5-minute Apgar scores, and preeclampsia, but not for abruptio placentae.

Table III displays the relative risk estimates with 95% confidence intervals for the adverse outcomes in Table

Table I. Characteristics of women during their index pregnancy by number of prior spontaneous abortions

Characteristic	*Women with no prior pregnancies (n = 3099)*	*Women with one prior spontaneous abortion (n = 2146)*	*Women with three or more prior spontaneous abortions (n = 638)*
Maternal age			
Mean (yr)	23.9	25.0	26.3
<20 yr (%)	22.8	15.5	10.3
20-24 yr (%)	34.8	34.6	30.6
25-29 yr (%)	26.3	28.9	30.6
>30 yr (%)	16.0	20.9	28.5
Smoker (%)	19.7	28.1	36.5
White (%)	87.6	89.5	91.2
Married (%)	72.4	76.2	75.9
Urban residence (%)	51.6	50.2	54.2
First-trimester prenatal care (%)	77.6	81.3	79.7

Table II. Number and percent of selected adverse outcomes in the index pregnancy by number of prior spontaneous abortions

	No prior pregnancy		*One prior spontaneous abortion*		*Three or more prior spontaneous abortions*	
Outcome	*No.*	*%*	*No.*	*%*	*No.*	*%*
Low birth weight	141	4.6	124	5.8	60	9.5
IUGR	133	5.0	94	4.9	41	6.4
Length of gestation <37 wk	220	7.8	174	8.7	63	10.8
34-36 wk	162	5.7	130	6.5	36	6.2
30-33 wk	47	1.7	26	1.3	19	3.2
<30 wk	11	0.4	18	0.9	8	1.4
Any malformation (except ductus)	53	2.0	36	2.0	21	3.8
Placenta previa	6	0.2	7	0.3	8	1.3
Membranes ruptured >24 hr	79	2.6	52	2.4	33	5.2
Breech	83	2.7	75	3.5	43	6.7
1 min Apgar score <6	206	6.7	175	8.2	63	9.9
5 min Apgar score <7	64	2.1	36	1.7	22	3.5
Preeclampsia	189	6.8	116	6.0	26	4.5
Abruptio placentae	25	0.9	19	0.9	5	0.8

II. Maternal age, smoking status, race, marital status, place of residence, and prenatal care were evaluated for confounding effects on all outcomes. Gestational age was evaluated as a potential confounder for all outcomes except IUGR. Only those variables that resulted in a meaningful change in the estimated relative risk were adjusted for in the analysis.

After adjustment for maternal age and smoking status, women with three or more prior spontaneous abortions had twice the risk of having a low-birth-weight infant (relative risk = 2.0, 95% confidence interval = 1.4 to 2.8) compared with that of women with no prior pregnancies. This association appears to primarily reflect an increased risk of preterm birth among women with recurrent abortions (relative risk = 1.5, 95% confidence interval = 1.1 to 2.1), rather than an increased risk of IUGR (relative risk = 1.2, 95% confidence interval = 0.9 to 1.7). The estimated relative risk for prematurity among women with three or more prior spontaneous abortions increased with increasing prematurity, from 1.2 (95% confidence interval = 0.8 to 1.8) for mild prematurity (34 to 36 weeks' gestation) to 2.7 (95% confidence interval = 1.0 to 7.3) for severe prematurity (<30 weeks' gestation).

Women with recurrent spontaneous abortions had an increased risk of delivering an infant with a congenital malformation (relative risk = 1.8, 95% confidence interval = 1.1 to 3.0). Ductus arteriosus was not included in congenital malformations because of its strong association with prematurity, which was already known to be associated with prior spontaneous abortions. Analysis by individual type of malformation was not possible because of small numbers. Analysis by major organ systems (genitourinary, cardiac, central ner-

Table III. Estimated relative risks and 95% confidence intervals* for selected adverse outcomes in index pregnancy by number of prior spontaneous abortions

Outcome	*One prior spontaneous abortion*		*Three or more prior spontaneous abortions*	
	Relative risk	*95% Confidence interval*	*Relative risk*	*95% Confidence interval*
Low birth weight	1.2	0.9-1.6	2.0	1.4-2.8
Intrauterine growth retardation	0.9	0.7-1.2	1.2	0.8-1.7
Gestation <37 wk	1.2	0.9-1.4	1.5	1.1-2.1
34-36 wk	1.2	0.9-1.5	1.2	0.8-1.8
30-33 wk	0.8	0.5-1.4	2.4	1.4-4.3
<30 wk	2.2	1.0-4.7	2.7	1.0-7.3
Any malformation (except ductus)	1.0	0.6-1.5	1.8	1.1-3.0
Placenta previa	1.6	0.5-6.0	6.0	1.6-22.2
Rupture of membranes >24 hr	1.0	0.7-1.4	1.8	1.2-2.9
Breech presentation	1.3	0.9-1.8	2.4	1.6-3.6
1 min Apgar score <6	1.3	1.0-1.6	1.2	0.8-1.6
5 min Apgar score <7	0.9	0.6-1.4	0.6	0.3-1.6
Preeclampsia	1.0	0.8-1.3	0.8	0.5-1.3
Abruptio placentae	1.2	0.6-2.3	1.2	0.4-3.1

*Low birth weight, IUGR, gestational age, and preeclampsia were adjusted for maternal age and smoking status. Placenta previa, prolonged rupture of membranes, and breech presentation were adjusted for maternal age and gestational length. One- and 5-minute Apgar scores were adjusted for maternal age, smoking status, and gestational length.

vous system, and limb defects) did not show a significantly elevated relative risk of malformation within any organ system.

Women with three or more prior spontaneous abortions also had elevated risks of placenta previa (relative risk = 6.0, 95% confidence interval = 1.6 to 22.2), prolonged rupture of membranes (relative risk = 1.8, 95% confidence interval = 1.2 to 2.9), and breech presentation (relative risk = 2.4, 95% confidence interval = 1.6 to 3.6), after adjustment for maternal age and gestational length. Additional adjustment for other variables, including smoking and prenatal care, did not alter these estimates.

After adjustment for maternal age, smoking status, and gestational length, women with recurrent spontaneous abortions had a relative risk of 1.2 (95% confidence interval = 0.8 to 1.6) of having an infant with a low 1-minute Apgar score. Women with one prior spontaenous abortion had a relative risk of 1.3 (95% confidence interval = 1.0 to 1.6) of having an infant with a low 1-minute Apgar score. There was no evidence of an increased risk of preeclampsia or abruptio placentae in either group.

Comment

Investigating the association between prior spontaneous abortion and subsequent adverse birth outcomes is complicated by the interrelationship between parity, gravidity, maternal age, smoking, number of prior spontaneous abortions,[14] and the interrelationship between adverse outcomes such as low birth weight, IUGR, prematurity, and low Apgar scores. The current study first simplified the examination of prior spontaneous abortion by limiting the study to women with prior spontaneous abortions who had experienced no other prior pregnancies. Women with one prior spontaneous abortion and women with three or more prior spontaneous abortions were examined separately because previous studies have suggested that women with three or more prior spontaneous abortions may be at higher risk for subsequent adverse pregnancy outcomes than women with only one prior spontaneous abortion,[2, 4, 6] The finding that smoking and maternal age were positively associated with the number of prior spontaneous abortions was expected, as both smoking[15, 16] and advanced maternal age[14, 17] have been associated with spontaneous abortion and women who reported smoking in the index pregnancies would be likely to have smoked during previous pregnancies. In this data set, maternal race, marital status, and prenatal care were similar among women with and without prior spontaneous abortions, and adjustment for these variables did not change the associations reported.

In the current study the adjusted relative risk of IUGR was 1.2 (95% confidence interval = 0.8 to 1.7) in women with three or more prior spontaneous abortions. This result is in contrast to a previous study that reported an increased unadjusted risk for severe IUGR in term infants (calculated relative risk = 2.2, 95% confidence interval = 1.6 to 2.9) in women whose previous

pregnancy ended in a spontaneous abortion when compared with women whose previous pregnancy ended in a live birth.[7]

The current study found only a slightly elevated adjusted relative risk of 1.2 (95% confidence interval = 0.9 to 1.4) for preterm birth in women with one prior spontaneous abortion but an adjusted relative risk of 1.5 (95% confidence interval = 1.1 to 2.1) for women with three or more prior spontaneous abortions. These results are similar to results reported from previous studies.[3, 4, 10]

Previous studies have suggested that prior spontaneous abortion is associated with an increased incidence of congenital malformation in a subsequent pregnancy.[9, 10] The rate of congenital malformation reported in the current study is less than that reported in previous studies.[10, 18] This difference probably reflects an underreporting of congenital malformations on the birth certificate. The current study found no evidence of an increased risk of congenital malformation in infants of women with one prior spontaneous abortion. However, women with three or more prior spontaneous abortions had a relative risk of 1.8 (95% confidence interval = 1.1 to 3.0) of having an infant with a congenital malformation. A larger study would be needed to evaluate the association between prior spontaneous abortions and specific malformation types.

A strong association was found in the current study between three or more prior spontaneous abortions and placenta previa (relative risk = 6.0, 95% confidence interval = 1.6 to 22.2) after adjusting for maternal age, smoking status, and gestational length. A previous study, which examined the combined risk of placenta previa and abruptio placentae in women with one prior spontaneous abortion, reported data that gave a calculated reltive risk of 2.3 (95% confidence interval = 0.7 to 7.9).[10] A more recent case-control study of 80 cases of placenta previa found calculated odds ratios of 1.5 for one prior spontaneous abortion (95% confidence interval = 0.5 to 4.1) and 4.9 (95% confidence interval = 0.3 to 88.4) for three or more prior spontaneous abortions.[6] The suggested explanation for this association was that curettage, commonly performed after a spontaneous abortion, may be a risk factor for subsequent placenta previa.

The current study found a moderate association between prolonged rupture of membranes and three or more prior spontaneous abortions, which was independent of gestational age and early prenatal care. This finding has not been previously reported. It is possible that this result reflects an association between delay in seeking medical care at the time of membrane rupture and other, unmeasured factors related to spontaneous abortion.

Another, apparently new finding is an elevated relative risk for breech presentation among women with three or more prior spontaneous abortions (relative risk = 2.4, 95% confidence interval = 1.6 to 3.6) after adjustment for maternal age, smoking status, and gestational length. The explanation for this finding is not apparent, although it could reflect underlying morphologic uterine abnormalities that predispose to both spontaneous abortion and breech presentation.

Women with one prior spontaneous abortion had a relative risk of 1.3 of having an infant with a 1-minute Apgar score <6, after adjustment for smoking, maternal age, and gestational age. This is less than the relative risk of 1.8 (95% confidence interval = 1.1 to 2.9) calculated from data reported in a previous study for a 1-minute Apgar score <6 among women with one prior spontaneous abortion.[10]

There are a number of limitations in the current study that should be noted. The use of birth certificate data to ascertain prior spontaneous abortion undoubtedly results in some misclassification of the number of prior spontaneous abortions. Because the information on prior spontaneous abortion is taken from the medical record, where it is usually recorded before the birth outcome, it is unlikely that prior spontaneous abortions were preferentially recorded for women with adverse outcomes. As long as such misclassification is not related to the outcomes of interest, the reported relative risk would be a conservative estimate of the true relative risk.

It is also possible that the associations reported may be the result of an unmeasured external variable, such as poor nutrition or alcohol or drug abuse, rather than a shared biologic mechanism. Socioeconomic status was not adjusted in the current study; however, mother's household income did not confound the associations in a pilot analysis with the same data. Moreover, adjustment for race, marital status, and first-trimester prenatal care, all of which would be expected to be related to socioeconomic status, did not affect any of the associations reported.

It is possible that the association between the adverse outcomes reported and three or more prior spontaneous abortions actually reflects an association with second-trimester spontaneous abortions,[19] which would be more common among women with three or more prior spontaneous abortions, rather than an association with the number of prior spontaneous abortions per se. This possibility could not be addressed in the current study because the data did not distinguish between first-trimester and second-trimester spontaneous abortions.

Multiple, nonexclusive causes have been suggested for spontaneous abortion, including chromosomal aberrations,[2, 20] maternal immunologic[21, 22] or hormonal[23] abnormalties, infection,[24] incompetent cervix,[25] and uterine malformations.[2] One or more of these factors may also result in an adverse birth outcome, creating an association between prior spontaneous abortion and

specific adverse birth outcomes. IUGR, for example, could plausibly result from immunologic, implantation, infection, or hormonal defects. Prematurity and IUGR have been associated with both maternal infection[26] and immunologic abnormalities.[21] Congenital malformations, while possibly related tc chromosomal aberrations, may also be the result of infection or of immunologic or hormone factors.[27] In spite of these complexities in the interpretation of the relation between prior spontaneous abortion and subsequent adverse birth outcomes, it is hoped that the results of the current study will provide clues to unraveling the etiology of spontaneous abortion.

The findings from the current study suggest that women whose first pregnancy ended in a spontaneous abortion are at little, if any, increased risk for an adverse outcome in a subsequent live-birth pregnancy. However, women with recurrent (three or more) spontaneous abortions and no other prior pregnancies are at increased risk for several adverse outcomes, including placenta previa, preterm delivery, and breech presentation. These results may be useful in guiding the clinical management of pregnancies in women with recurrent abortions.

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Prolonged effects of a novel, low-dosage continuous progestin–cyclic estrogen replacement program in postmenopausal women

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OBJECTIVE: Our objective was to ascertain long-term tolerability and effects of a novel, low-dosage continuous progestin–cyclic estrogen regimen on vasomotor flushing, vaginal bleeding patterns, bone density, and plasma lipoprotein lipids.
STUDY DESIGN: Eighteen postmenopausal women with climacteric symptoms received low doses of *dl*-norgestrel (0.075 mg/day) continuously and 17β-estradiol (1 mg/day) intermittently (25 of 28 days) for 3 years. The results were compared with baseline values.
RESULTS: The hormonal regimen was well tolerated. Vaginal bleeding, vaginal spotting, and vasomotor flushing occurred in only 0.4%, 4.2%, and 7.7% of cycles, respectively. Bone density was stable. Mean fasting plasma total cholesterol concentration fell 19%, low-density lipoprotein cholesterol 23%, high-density lipoprotein cholesterol 13%, and triglycerides 31% ($p < 0.01$) over the 3-year period, while the ratios of low-density lipoprotein cholesterol/high-density lipoprotein cholesterol and total cholesterol/high-density lipoprotein cholesterol decreased by 14% and 10%, respectively.
CONCLUSION: Long-term compliance with female hormonal replacement is feasible once vaginal bleeding is essentially eliminated, allowing for potentially better prophylaxis against coronary heart disease, osteoporosis, endometrial cancer, and stroke. (Am J Obstet Gynecol 1992;166:117-21.)

Key words: Hormonal replacement therapy, cholesterol, triglycerides, osteoporosis, endometrium

Estrogen deficiency has been implicated in causing osteoporosis, vasomotor instability, insomnia, depression, dyspareunia, and urinary stress incontinence.[1] Twenty-five percent or more of women of postmenopausal age are sufficiently symptomatic of estrogen deprivation to warrant replacement on that basis alone, and progestin replacement may be warranted in more.[2, 3] Whereas two centuries ago <30% of women lived long enough to reach menopause, currently 90% do, including >30 million American women with an average postmenopausal life expectancy of 28 years.[4] In spite of the permanency of ovarian hormone deficiency, only about 2 to 3 million American women currently take estrogens,[5] and only 19% of physicians would prescribe them for ≥3 years.[6] When ovarian secretion of 17β-estradiol and progesterone declines, the concentration of serum cholesterol rises,[7] and women lose their relative immunity (as compared with men) from ischemic heart disease.[8] They then also experience increases in the incidence of endometrial, ovarian, and breast cancer[9] and increased morbidity and mortality from osteoporosis-related fractures.[10]

Prolonged estrogen therapy that is unopposed by a balance of natural progesterone or synthetic progestin can induce proliferative changes in the lining of the uterus, which can progress to hyperplasia, adenomatous hyperplasia, and frank endometrial adenocarcinoma.[11] The relative risk of 1.7 to 8.0 for endometrial cancer can be reduced below 1.0 by intermittently administering progestin together with estrogen.[12] However, this leads to scheduled induction of withdrawal bleeding in 90% of women with uteri in situ.[13] During the interruption of therapy, menopausal symptoms frequently relapse, and recurrence of bothersome, painful, and at times heavy vaginal bleeding induces anxiety about cancer and return of fertility. These concerns have hampered the long-term use of female hormones for the prevention of osteoporosis, coronary heart disease, and stroke.[3, 14] The purpose of the present pilot study was to assess the efficacy and long-term acceptability of a novel low-dosage progestin-estrogen combination for hormonal replacement in postmenopausal women.

Material and methods

Twenty-one women (mean age 53 ± 2 years, mean height 162 ± 2 cm, mean weight 64 ± 3 kg) were re-

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Table I. Effects of prolonged combined treatment with 17β-estradiol and *dl*-norgestrel on fasting plasma lipoprotein concentrations*

	1 yr (n = 12)		*2 yr (n = 18)*		*3 yr (n = 13)*	
	C (mg/dl)	*E_2 + Ng (mg/dl)*	*C (mg/dl)*	*E_2 + Ng (mg/dl)*	*C (mg/dl)*	*E_2 + Ng (mg/dl)*
Total cholesterol	254 ± 19	222 ± 12†	244 ± 15	202 ± 10‡	242 ± 14	196 ± 10‡
VLDL cholesterol	14 ± 2	11 ± 2	14 ± 1	13 ± 2	13 ± 1	14 ± 2
LDL cholesterol	184 ± 21	166 ± 12	177 ± 14	141 ± 10‡	175 ± 14	135 ± 10‡
HDL cholesterol	53 ± 3	49 ± 4	52 ± 2	47 ± 2	53 ± 2	46 ± 2§
Total trigylcerides	179 ± 17	134 ± 12‖	158 ± 14	123 ± 15¶	159 ± 14	110 ± 10‡
VLDL trigylcerides	74 ± 11	44 ± 7§	70 ± 8	51 ± 9§	68 ± 8	47 ± 7#
Ratio of LDL cholesterol to HDL cholesterol**	3.6 ± 0.6	3.3 ± 0.4	3.5 ± 0.5	3.1 ± 0.3	3.6 ± 0.5	3.1 ± 0.3
Ratio of total cholesterol to HDL cholesterol††	5.1 ± 0.7	4.5 ± 0.5	4.9 ± 0.5	4.4 ± 0.3	4.9 ± 0.5	4.4 ± 0.2

C, Control; *E_2*, 17β-estradiol; *Ng*, *dl*-norgestrel; *VLDL*, very-low-density lipoprotein.

*Values (mean ± SEM) for total cholesterol, very-low-density lipoprotein cholesterol, LDL cholesterol, HDL cholesterol, total triglycerides, and very-low-density lipoprotein triglycerides are for the same subjects during baseline control and after 1 year, 2 years, and 3 years of 17β-estradiol and *dl*-norgestrel treatment.

†Significantly different from control, $p < 0.02$.

‡Significantly different from control, $p < 0.001$.

§Significantly different from control, $p < 0.01$.

‖Significantly different from control, $p < 0.005$.

¶Significantly different from control, $p < 0.05$.

#Significantly different from control, $p < 0.025$.

**Values for 5th, 50th, 75th, and 95th percentiles from the Framingham study are 1.3, 2.7, 3.5, and 5.1, respectively.[18]

††Values for 5th, 50th, 75th, and 95th percentiles from the Framingham study are 2.6, 4.2, 5.2, and 7.3, respectively.[18]

cruited from University Hospital Outpatient Clinics. Fifteen subjects had uteri in situ. Each subject met the following criteria: amenorrhea for at least 6 months, serum follicle-stimulating hormone level >70 mIU/ml (mean 129 ± 5 mIU/ml), typical menopausal symptoms, and absence of contraindications for estrogen therapy.[3] Three subjects had hypercholesterolemia (395, 350, and 294 mg/dl) and two had hypertriglyceridemia (272 and 282 mg/dl).[15] The nature and possible consequences of the studies were explained to each subject and informed consent was obtained.

Initial examination consisted of a complete medical history (including menopausal symptom assessment), physical examination, measurement of serum electrolyte levels, creatinine, blood urea nitrogen, albumin, globulin, calcium, phosphate, uric acid, total bilirubin, follicle-stimulating hormone, luteotropic hormone, alkaline phosphatase, alanine transaminase, γ-glutamyl transpeptidase, white blood cell count, hematocrit, and a chest roentgenogram. A dietitian instructed each subject before baseline lipid testing to maintain her weight and diet, except that the diet should contain <500 mg cholesterol per day. However, exercise level, intake of ethanol, and consumption of cigarettes were not monitored systematically.

17β-Estradiol was supplied by Bristol Laboratories of Canada, Ottawa, and *dl*-norgestrel by Wyeth Ltd., Downsview, Ontario. Compliance with the hormonal regimen was monitored by recording every pill taken, along with the daily record of flushing and vaginal spotting or bleeding. Mean baseline values for fasting plasma lipid and lipoprotein lipid fraction levels (after a 12-hour fast) and blood pressure were based on the average of three determinations from each subject after a 4-week washout period that followed administration of similar doses of each of 17β-estradiol for 8 weeks and *dl*-norgestrel for 8 weeks or 17β-estradiol and *dl*-norgestrel for 24 weeks in the antecedent randomized study, in which all of the present subjects had participated. After baseline testing each subject was placed on a regime of *dl*-norgestrel (0.075 mg/day) continuously and 17β-estradiol (1 mg/day) intermittently (25 of 28 days).

Eighteen of the 21 subjects who volunteered completed the 3-year study. One subject was excluded early because of noncompliance, another because of intercurrent illness, and one moved away in the third year. Nurses coordinated the clinic visits and testing and measured blood pressure at each semiannual visit. Fasting plasma concentrations of lipids and lipoprotein fractions and the bone density of the left radius were measured annually and blood pressure and weight

Table II. Effects of prolonged combined treatment with 17β-estradiol and *dl*-norgestrel on relief of postmenopausal symptoms*

	Cycles free of individual symptoms for each period of treatment (%)							
	Year 1		*Year 2*		*Year 3*		*Years 1-3*	
Symptom	*C*	*E_2 + Ng*	*C*	*E_2 + NG*	*C*	*E_2 + Ng*	*C*	*E_2 + Ng*
Flushing	22.2	92.6†	22.2	92.7†	22.2	91.4†	22.2	92.3†
Spotting	100.0	97.7	100.0	94.7	100.0	95.1	100.0	95.8
Bleeding	100.0	100.0	100.0	100.0	100.0	98.9	100.0	99.6

C, Control; E_2, 17β-estradiol; *Ng*, *dl*-norgestrel.

*Symptoms were recorded daily by 15 subjects for each of three 28-day cycles of placebo control, and for up to 39 cycles (3 years) of 17β-estradiol and *dl*-norgestrel treatment. However, the placebo control value for spotting was taken as 100.0% because in practice spotting tended to occur during placebos after active hormone administration (25% to 50% of cases).

†Significantly different from control, $p < 0.001$.

semiannually. Lipids and lipoprotein fractions were determined as previously described.[16] Comparisons between paired samples were carried out with either the two-tailed Student *t* test or χ^2 analyses according to Steel and Torrie.[17]

Results

Plasma lipids and lipoproteins. Fasting plasma lipid levels decreased significantly and progressively below the baseline control during 17β-estradiol and *dl*-norgestrel therapy (Table I). There was a trend toward greater lowering of low-density lipoprotein (LDL) cholesterol as a function of initial concentration, but the coefficient of correlation failed to be statistically significant ($r = 0.42, 0.05 < p < 0.10, n = 19$). The mean ratio of plasma LDL cholesterol/high-density lipoprotein (HDL) cholesterol, an index of atherosclerotic risk, decreased from 3.6 ± 0.5 to 3.1 ± 0.3 at 3 years (14%, $0.10 < p < 0.20$), whereas that of another indicator, the mean ratio of total cholesterol/HDL cholesterol, decreased from 4.85 ± 0.46 to 4.37 ± 0.24 (10%, $0.10 < p < 0.20$).

Symptoms (flushing, spotting, and bleeding). Virtually complete relief of flushing was achieved. Fourteen of the 15 subjects with uteri did not detect any type of flushing over the third year (95.8% of cycles free, Table II). However, one subject had frequent mild atypical sweating in all but two cycles (average of 11 ± 1 days per 28-day cycle). The findings were atypical of flushing, but the subject (166 cm tall, 94 kg) construed them as such.

Vaginal spotting not necessitating protection was not experienced by 11 of the 15 subjects with uteri in situ and was absent in 95.8% of all cycles ($n = 15$). (Unpublished findings of endometrial biopsy specimens during the antecedent randomized study showed atrophic or scanty endometrium in 10 subjects who underwent biopsy before treatment and in nine of the same subjects who underwent biopsy after receiving 17β-estradiol and *dl*-norgestrel for 24 weeks [six cycles]; in one of these 10 subjects there was weak evidence of proliferative endometrium after treatment.) Over the 3-year period two subjects reported spotting on 1 day only, one subject on 1 day of each of two cycles, and a third subject on 2 days during only one cycle. Only one subject had frequent spotting (average of 2 days per cycle in 17 of 37 cycles, followed by one episode of vaginal bleeding during each of two further cycles). The other 14 subjects with intact uteri had no vaginal withdrawal bleeding, except for one subject who transiently failed to take medication as prescribed. Overall, vaginal bleeding was virtually eliminated (in 99.6% of cycles).

Bone density. Forearm bone density was not significantly changed from baseline control (0.67 ± 0.02) after 1 year (0.68 ± 0.02 gm/cm²), 2 years (0.69 ± 0.02), or 3 years (0.68 ± 0.02 gm/cm²) of therapy ($n = 17$, $p > 0.5$).

Blood pressure. The significant mean increase in body weight of 1.7 kg over the 3-year study (67.4 ± 3.2 vs 65.7 ± 3.1 kg, $p < 0.01$) was associated with a slight rise in diastolic blood pressure above baseline control. This was statistically significant at 2 years (79 ± 2 vs 75 ± 1 mm Hg, $n = 19, p < 0.05$) but not at three years (79 ± 2 vs 76 ± 2, $n = 18$). The associated slight rise above baseline control in systolic blood pressure after 2 years (137 ± 4 vs 124 ± 4 mm Hg, $n = 19$) was also maintained after 3 years (135 ± 3 vs 125 ± 4 mm Hg, $n = 18, p < 0.025$).

Comment

The current studies demonstrate the feasibility of long-term patient compliance with low-dosage 17β-estradiol and *dl*-norgestrel hormonal replacement therapy, which virtually eliminated menstrual spotting and bleeding that constitute the main obstacle to continu-

ance of therapy.[14] Recurrence of flushing is frequently reported during the estrogen-free period with most postmenopausal hormone replacement regimens (7 estrogen-free days of a total of 28); however, this was not observed when 17β-estradiol was interrupted for only 3 days per cycle and *dl*-norgestrel was given continuously (Table II). The finding of effective control of vasomotor symptoms and vaginal bleeding through the combined use of continuous low-dosage progestin and cyclic estrogen is consistent with similar results with the use of continuous low dosages of conjugated equine estrogens and medroxyprogesterone acetate[19, 20] or comparably low doses of ethinyl estradiol (about 5 μg daily) combined with oral contraceptive dosages of norethindrone acetate.[21] Prior exposure to progestin in the antecedent study explains the low incidence of vaginal bleeding or spotting in the first year. The 3-day-per-cycle interruption of estrogen administration in this study has the benefit of reducing total estrogen dosage by about 10% and thereby reducing the occurrence of mastodynia (recognized estrogen side effect) without any detectable increase in vasomotor symptoms. Interruption of estrogen may also play a role in reducing breakthrough bleeding.

Long-term therapy could yield important benefits for prevention of ischemic heart disease, the major cause of death (and a major source of disability) among menopausal and postmenopausal women, which causes four to five and a half times more deaths among them than do cancer of the breast and endometrium combined.[22, 23] The 23% mean decrease in LDL cholesterol in this study exceeded the 13% decrease in HDL cholesterol, yielding a favorable decrease in the ratios of LDL cholesterol/HDL cholesterol and total cholesterol/HDL cholesterol, which are important indicators of risk of coronary heart disease. The androgenic effects of 19-nortestosterone-derived *dl*-norgestrel likely account for the observed reduction in HDL cholesterol but could also play a role in the significant reduction in LDL production and concentration obtained with combined low-dosage *dl*-norgestrel and 17β-estradiol.[16] Continuous estrogen-progestin combinations with various low doses of ethinyl estradiol and oral contraceptive dosages of norethindrone acetate also minimize menstrual spotting and bleeding, though no significant changes in serum lipids were reported.[21]

In contrast to the life-threatening hyperlipidemia that can develop during estrogen therapy of hypertriglyceridemia subjects,[24] a significant lowering of triglyceride levels was observed with combined 17β-estradiol and *dl*-norgestrel (Table I). Although estrogens are often contraindicated in patients with hypertriglyceridemia,[24] the combination of 17β-estradiol and *dl*-norgestrel could sometimes be beneficial under such circumstances. The reduction of triglyceride-rich lipoprotein levels may be useful for the prophylaxis of coronary heart disease, given the evidence implicating them in its genesis.[25]

We found no change in radial bone density over 3 years, in contrast to the expected reduction which is detectable within a year in postmenopausal women not receiving estrogen. However, vertebral bone density would likely have been a more sensitive test of bone loss. The risk of bone fracture is known to be reduced by prophylactic estrogens.[10]

The current study demonstrates the feasibility of using a low-dosage 17β-estradiol and *dl*-norgestrel postmenopausal replacement program for 3 years to relieve vasomotor symptoms, eliminate vaginal bleeding, maintain radial bone mass, and reduce lipoprotein-associated risk of coronary heart disease and nonhemorrhagic stroke by decreasing plasma LDL and triglyceride levels and the ratios of LDL cholesterol/HDL cholesterol and total cholesterol/HDL cholesterol.[3]

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Attitudes toward reproduction in a nonpatient population

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OBJECTIVE: A basic question to be answered in an attempt to understand human reproductive behavior as it manifests itself in clinical practice is, why do people want to have children?

STUDY DESIGN: A psychometric instrument for the assessment of reproductive attitudes was developed. A total of 746 men and women at 20, 30, and 40 years of age, who were randomly selected from a nonpatient population, answered. Data were analyzed by factor analysis. Reproductive profiles were constructed.

RESULTS: The two most important factors in both sexes were "children as existential satisfaction" and "children as lack of freedom," indicating a basic conflict. The third most important factor in both sexes was "the importance of one's own parents as examples in parenthood," supporting earlier findings that reproductive conflicts are transmitted from one generation to another. Reproductive profiles were uniform in the different age groups.

CONCLUSION: The element of ambivalence may be a clue to a deeper understanding of human reproductive behavior. (AM J OBSTET GYNECOL 1992;166:121-6.)

Key words: Attitudes toward parenthood, child wish, factor analysis, reproductive ambivalence

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Present-day attitudes toward sexuality, the availability of effective and reliable contraceptives, legislation on abortion on request, and technical developments to hinder or promote fertility may give the superficial impression that whether to have a child is a decision based solely on rational choices.[1] However, the strong emotional reactions evoked in society by these subjects[2] and the multitude of physical and mental reactions linked to contraception,[1, 3, 4] wanted and unwanted

Table I. Number of distributed and returned questionnaires in different age groups, women and men

Age (yr)	Women			Men		
	Distributed (No.)	Returned		Distributed (No.)	Returned	
		No.	%		No.	%
20	176	141	80	173	101	58
30	172	143	83	153	111	73
40	160	132	83	167	117	70
TOTAL	508	416	82	493	329	67

pregnancies,[5, 6] and infertility[7, 8] all tend to compound the complexity of this seemingly simple matter. In clinical practie we often find ourselves as observers of a reproductive drama. Adaptational difficulties in the spheres of reproduction, motherhood, and femininity have been suggested to be factors underlying various somatic and mental reproductive disorders.[6] In modern Western societies, traditional norms for reproductive behavior are rapidly changing, which will probably render role adaptation more complicated. One important basic question to answer seems to be: Why do people want to have children[1]?

The psychoanalytic and psychologic literature on motives for having or not having children is extensive.[5, 9, 10] Nevertheless, there are few investigations based on larger populations, and evidently such studies raise methodologic problems.[10] Most previous attempts in this field have focused mostly on specific groups of patients or pregnant women or on students.[11-13] Although the husband's attitudes are most important for his wife's well-being during pregnancy,[13] little has been written on the male wish for progeny. Clearly, it would be valuable to obtain more general information on the matter from nonpatient populations. How do ordinary men and women approach the issue of reproduction and its vicissitudes during the different phases of reproductive life? In Sweden the unique national registration system provides possibilities for population-based investigations. We report here the results of a large-scale study on attitudes toward reproduction among men and women of fertile age.

Material and methods

A psychometric instrument for the assessment of reproductive profiles in men and women was devised.[14] The inventory comprises 53 items, the content of which was based on previous research,[7, 12] psychologic and psychoanalytic literature,[5, 9, 10] and clinical experience (M.W.). Negative and positive statements toward pregnancy and parenthood are balanced. They focus on four main reasons for wanting a child, i.e., philosophic or existential, sociocultural, interpersonal, and intrapsychic. "Wish for pregnancy" and "wish for a child" are considered as two distinct and not necessarily related desires. Seven of the 53 items are sex specific. The items are to be answered in a five-point response format according to Likert.[15] The instrument has earlier been found very reliable (Cronbach's α 0.7888).[14]

The population studied comprised inhabitants within the catchment area for Umeå University Hospital. Umeå is an average-size city on the northern Baltic coast of Sweden. Originally a center in a rural area with a strong religious tradition, it has developed into a modern center for education, and culture. The population of 90,000 is comparatively young (mean age 36 years) and is growing by a thousand persons per year.

Men and women were randomly selected from the national registration records. Representative samples of the population in three different phases of fertile life were created.

A questionnaire was mailed with an accompanying letter explaining the purpose of the study to 1001 persons, 20, 30, or 40 years old. Two reminders were sent to nonresponders. Data from completed forms were computerized and analyzed by factor analysis type PA 2 with oblique rotation.[16] Men and women were analyzed separately. Factor analysis was used to reduce the large number of variables to describe a structure in a complicated pattern. The number of factors in this work was chosen from the interpretability of their content. For each sex six factors were extracted. The mean group response to the selected factors was assessed. For each factor an index, I, was calculated according to the formula:

$$I = x_{v1} \cdot f_1 + x_{v2} \cdot f_2 + \ldots x_{vn} \cdot f_n$$

where x is the average group response to item V, 1, 2 . . . n indicates the rank of each item within the factor, and f represents the value of the factor loading. Only items with a factor loading >0.38 were included. The number of items within each factor ranged between 2 and 10.

For each factor an I_{min} and an I_{max} were calculated, and a reproductive profile was constructed where $I_{min} = 0$ and $I_{max} = +10$. Indices were produced for each age group in both sexes.

Table II. The six most important factors achieved by factor analysis of 53 items answered by 416 women

Factor	*Variance (%)*	*Content*	*Important items*	*Factor loading*
1	33	Children—existential satisfaction	A child is a confirmation of a love relationship.	0.59
			Having children is a way to communicate your knowledge and life experience to future generations.	0.58
			The children around you enhance your faith in the future.	0.58
			It is the greatest happiness in life to watch a child grow up.	0.55
2	26	Children—lack of freedom and a burden	You become afraid to have children when you see how unfree parents of small children are.	0.63
			When you have children you can no longer travel around the world as you would like to.	0.56
			You lose your freedom when you have children.	0.54
3	14	Importance of own parents as an example	As a parent I want to be like my own mother was toward me.	0.76
			As a parent I don't want to be like my own mother was toward me	−0.73
4	11	Importance of the child's sex	To me it is important to have a daughter.	−0.70
			To me it is important to have a son.	−0.61
5	10	Children—the meaning of love and life?	You may feel like a perfectly complete woman without having experienced a pregnancy.	0.66
			A woman cannot feel quite satisfied in life until she has experienced a pregnancy.	−0.50
			A love relationship is not complete until you have a child together.	−0.42
6	7	The impact of ambivalence	Difficulties in tolerating contraceptives are expressions of a subconscious child wish.	0.65
			"Negligence" about contraception is due to a subconscious desire to become pregnant.	0.65

The relative importance of each factor is denoted by the percentage of variance.

Results

Of the 1001 forms distributed, 746 were completed and returned. Thus the average response was 75% (women 82%, men 67%). The response rates in different age groups are illustrated in Table I. The six factors are described in Tables II and III. Their relative importance is expressed by the percentage of variance, and a few items with a high factor loading are given. The overall response pattern was strikingly similar between men and women.

Thus the two most important factors for both sexes were "children as existential satisfaction" and "children as lack of freedom." Together these two factors were found to account for about 60% of the variance. Whereas women ranked "children as existential satisfation" the most important factor, "children as lack of freedom" was considered relatively more important by men. There were also other subtle differences between male and female attitudes, as indicated by the slightly different formulations of the factors. Restriction of freedom was more concretely expressed in the items highly loaded by the women, whereas the men had a more general and abstract approach to this issue. Having children as a way to find existential security in life had a high factor loading in the men's factor 2, whereas existential satisfaction expressed as "a child is a confirmation of a love relationship" appealed to the women.

Factor 3 concerns "the importance of one's own parents as examples in parenthood" and evoked virtually identical responses from both sexes. This factor comprised four items, all of which are about whether one wants (or does not want) to be like one's mother and

Table III. The six most important factors achieved by factor analysis of 53 items answered by 329 men

Factor	*Variance (%)*	*Content*	*Important items*	*Factor loading*
1	36	Children—lack of freedom	You lose your freedom when you have children.	0.61
			You become afraid to have children when you see how unfree parents of small children are.	0.60
			It is difficult to realize the meaning of life when you have parental responsibility.	0.55
2	25	Children—existential consolation and satisfaction	Having children is a way to find security in life.	0.57
			The children around you enhance your faith in the future.	0.56
			It is the greatest happiness in life to watch a child grow up.	0.53
3	14	Importance of own parents as an example	As a parent I want to be like my own father was toward me.	−0.79
			As a parent I don't want to be like my own father was toward me.	0.75
4	10	Fecundity—gift or coercion	It is selfish not to have children.	−0.43
			A love relationship might be complete without children.	0.42
5	11	Importance of the child's sex	To me it is important to have a daughter.	−0.59
			To me it is important to have a son.	−0.51
6	7	The impact of ambivalence	Difficulties in tolerating contraceptives are expressions of a subconscious child wish.	−0.65
			You become afraid to have children of your own if you got on badly with your mother.	−0.47
			"Negligence" about contraception is due to a subconscious desire to become pregnant.	−0.44

The relative importance of each factor is denoted by the percentage of variance.

father, respectively, when one becomes a parent. The men ranked their fathers as more important than their mothers; conversely, the women ranked their mothers as more important than their fathers. In the women's factor 3 there was an additional fifth item with a comparatively low factor loading, 0.42. This fifth item has the wording, "I was a burden to my parents."

The men's factor 4, "fecundity, gift or coercion," was not identified among the women, and likewise the women's factor 5, "children as a means for achievement," was not found in the male pattern. The importance of the child's sex emerged as factor 4 in the women's analysis and as factor 5 among the men with the identical two items. Factor 6 in both sexes could be comprehended as "the impact of ambivalence." The perception of the content of this factor may differ between men and women, as exemplified in the items in the tables.

The mean group attitudes to the dominant factors were calculated. The resultant reproductive profiles for the different age and sex groups are shown in Figs. 1 and 2.

There was a striking similarity between the different age groups. When calculating corresponding indices for men to the women's factors and vice versa, a strong conformity between the sexes was also found.

Comment

The current results indicate a high degree of concordance in reproductive attitudes between women and men and between young and middle-aged people in different phases of their fertile life. We feel that what we have perceived are collective attitudes to pregnancy, children, and parenthood rather than individual private motives. The impact of collective attitudes on the motivation for reproduction in individuals has been demonstrated previously,[9] and certainly the results could be valid also for individual patients in clinical practice. These attitudes are dominated by two conflicting forces, i.e., the view of children as an existential satisfaction and children as a restriction of freedom and a burden. These findings are in line with an earlier study by Gaughran et al.[17] The focus of their items was more of an ideologic nature, but nevertheless the two most important factors "concern with population growth" and "large family ideology" express the same basic conflict. Indeed, this antagonism seems to create the basis of the reproductive drama.

Although the men's and women's attitudes were quite similar in most respects, there were still interesting differences. In Sweden, and possibly in many Western countries, women provide most of the practical and daily care for their children, often at the cost of a professional career. Men seem to feel their responsibility is to provide for the economic well-being of the family. This may be reflected in the differences in the content of the factor "children as a burden." The sequence of order where women place the factor "children as existential satisfaction" first, whereas men place it second, may reflect a convention where women are less allowed than men to reject parenthood. An alter-

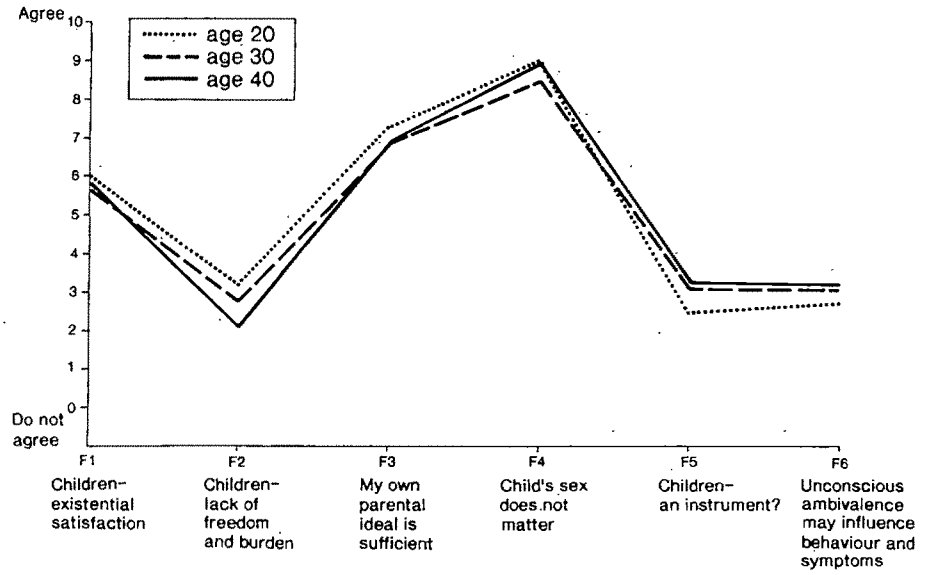


Fig. 1. Mean group attitudes toward six dominant factors about reproduction in 20-, 30-, and 40-year-old women.

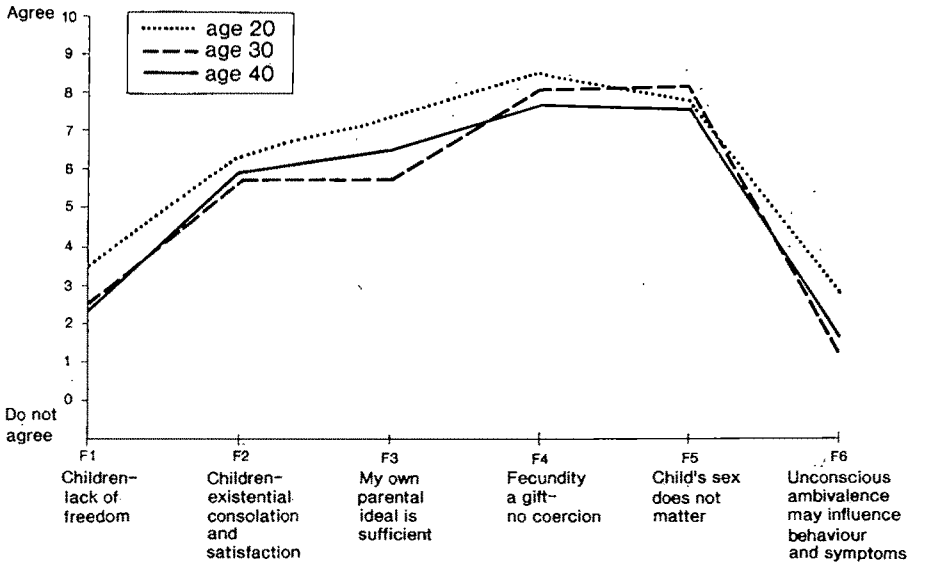


Fig. 2. Mean group attitudes toward six dominant factors about reproduction in 20-, 30-, and 40-year-old men.

native explanation might be that the biologic rhythm of women and men differs fundamentally—women's cyclicity perpetually and concretely reminds them of their reproductive potential.

The strong third factor dealing with the significance of one's own parents as an example in parenthood might be especially important for individual choice and behavior. Many authors[6, 18] have described how reproductional conflicts are transmitted from one woman-generation to another. This knowledge could be useful in clinical work with patients where reproductive conflicts are expressed, for example, as intolerance toward different contraceptives or repeated unwanted pregnancies. These women might benefit from consultations where realistic needs and possible hereditary conflicts are elucidated.

Little is known about reproductive conflicts in men. Previous studies have demonstrated that their attitudes markedly influence the well-being of their wives during pregnancy.[13, 18] Here the third factor about the significance of one's own parents as an example was similar in men and women, thus supporting the assumption that there is little difference between the sexes in this respect. To what extent male reproductive conflicts are transmitted through generations should be a theme for further research.

Although most subjects in our study answered that the child's sex was immaterial, this question was sufficiently loaded to emerge as a distinct factor in the analysis. In interviews with women about children, family, and work published by Statistics Sweden (1985),[19] mothers of two children were more inclined to have a third child if the first two children were of the same sex, regardless of whether they were boys or girls. In parents-to-be, Kihlbom et al.[18] found that the sex of the future child was extremely important, although there was a strong pressure to love the child whatever its sex. However, negative reactions were occasionally evoked when the child was born with the "wrong" sex.

When traditional roles are in a state of transition, it is noteworthy that the two most loaded items in the women's factor 5 concerned whether a pregnancy is necessary to make a woman feel a "real woman." The importance of sexual identity conflicts has been discussed previously.[3] Indeed, some insecure women may "want motherhood more than the child."[20] It is no doubt of clinical importance to perceive these attempts to buttress a fragile identity. The topic is subtle and delicate and respectful, empathic, and supportive listening in the consulting room is important.

Although most men and women in this population denied that ambivalence should exert any influence on either conscious or subconscious behavior (Figs. 1 and 2), the topic was loaded and found to constitute factor number 6. This finding again illustrates the basic conflict between opposing forces. In fact, ambivalence seems to be integral to collective attitudes toward reproduction. This may be a clue to a more complete understanding of the reproductive drama.

Our efforts to help women and men to enjoy sexuality without fear of unwanted pregnancies and to assist them when child wish is expressed may be even more successful if we recognize the element of ambivalence in human reproductive behavior.

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Acute posttraumatic fetal anemia treated with fetal intravascular transfusion

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Fetal trauma resulting in acute anemia after maternal blunt abdominal trauma is a rare but potentially lethal condition. We recently managed such a case with the use of fetal intravascular transfusion. (Am J Obstet Gynecol 1992;166:127-9.)

Key words: Fetal trauma, fetal anemia, fetal intravascular transfusion

Maternal trauma during motor vehicle accidents is considered a significant contributor to perinatal morbidity and mortality in the United States. We report on the use of fetal intravascular transfusion in the management of posttraumatic fetal anemia in the early third trimester.

Case report

A 28-year-old woman, gravida 5, para 3-0-1-2, was transported to our institution at 27.5 weeks' gestation because of suspected fetal distress after a motor vehicle accident in which her abdomen hit the steering wheel. There was no history of drug or alcohol use. The prenatal course and medical history were unremarkable. On admission, the patient was alert, cooperative, and in no distress. Vital signs were stable. Abdominal examination revealed no ecchymoses, mild right upper-quadrant pain, and a soft uterus. Fundal height was 28 cm. There was no evidence of ruptured membranes, vaginal bleeding, or labor. Ultrasonographic examination did not show any fetal or placental anomalies. Results of umbilical artery Doppler studies were normal. External fetal heart monitoring demonstrated a persistent tachycardia of 170 to 180 beats/min with a sinusoidal pattern and no decelerations (Fig. 1). Biophysical profile revealed no fetal breathing, but adequate tone, movements, and amniotic fluid were present. Results of routine admission laboratory and coagulation studies were normal. Kleihauer-Betke test result was negative (<0.1% fetal cells), Rh status was positive, and antibody screening test result was negative. Urine toxicology screen was negative.

The fetus was monitored continuously, with the same

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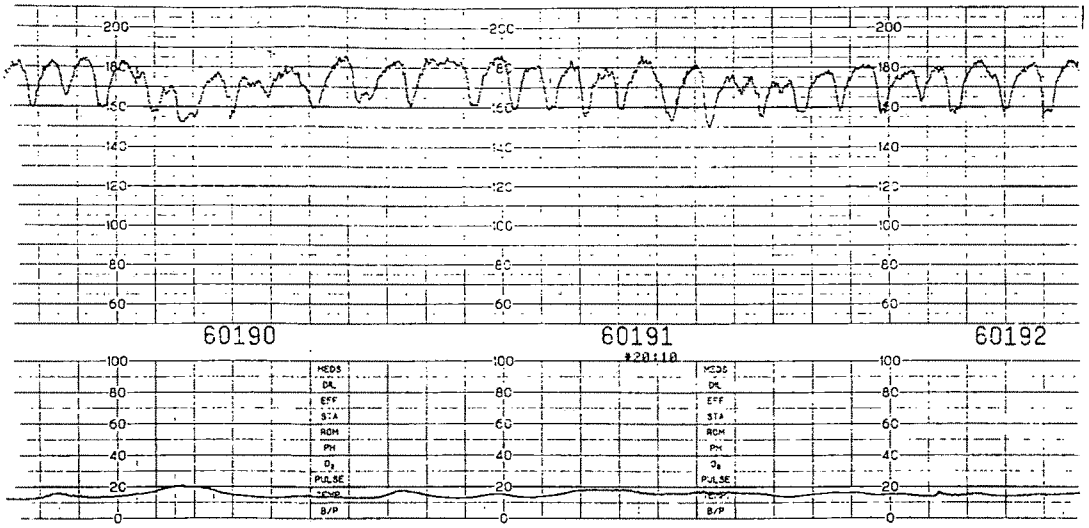


Fig. 1. Fetal monitoring strip before intravascular transfusion.

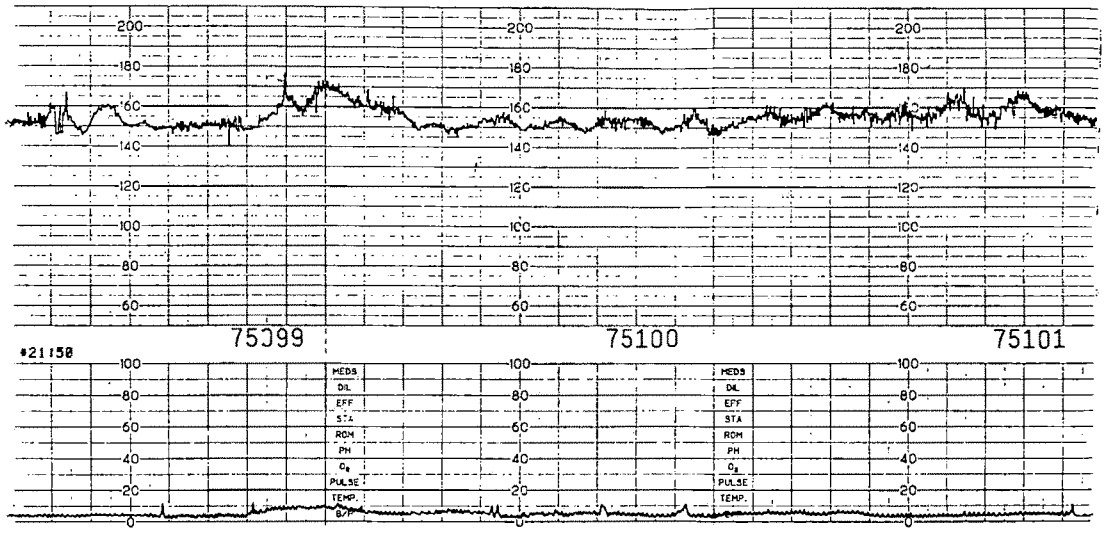


Fig. 2. Fetal monitoring strip after intravascular transfusion.

heart rate pattern always maintained. Serial biophysical profiles were not suggestive of hypoxia. To further investigate the cause of the abnormal heart rate pattern, amniocentesis and cordocentesis were performed. Amniocentesis revealed bloody amniotic fluid. Cordocentesis revealed a hematocrit of 17%, mean cell volume of 110.5, pH 7.4, Pco_2 38.0 mm Hg, bicarbonate 21.6 mEq/L, oxygen saturation 72.2%, and Kleihauer-Betke test >99% fetal cells. Subsequently, negative screens for toxoplasma and cytomegalovirus immunoglobulin M and normal female karyotype were reported. An intravascular fetal transfusion was performed with 70 ml packed, washed, irradiated, cytomegalovirus-negative, O-negative red blood cells. A posttransfusion hematocrit was not obtained because the needle dislodged and a second attempt at needle insertion failed. After the transfusion, fetal heart rate pattern showed a normal baseline and gradual disappearance of the sinusoidal pattern (Fig. 2). Daily fetal surveillance revealed reactive nonstress tests and normal biophysical profiles. The patient was discharged on the sixth hospital day. Further antepartum surveillance included fetal movement counts, nonstress testing, and ultrasonographic evaluations for growth.

The patient was admitted at 37 weeks with ruptured membranes. After augmentation of labor with oxytocin she was delivered vaginally without complications of a 3905 gm female infant with Apgar scores of 8 at 1 minute and 9 at 5 minutes. Cord hematocrit was 50%. The placenta had an area of old infarction covering 25% of the fetal surface. The neonate was diagnosed as having microcephaly (head circumference <3 SD). Results of neurologic evaluation were basically normal, and antibody titers for toxoplasma and cytomegalovirus were negative. Computed tomographic scan of the brain revealed evidence of parenchymal atrophy from a suspected resolving old hemorrhagic process in the left parietooccipital area. After initial problems with feeding, the baby was discharged on the sixth postpartum day.

Comment

Fetal injuries resulting from maternal blunt abdominal trauma may be due to abruptio placentae, cord rupture, fractures, intracranial hemorrhage, prematurity, uterine rupture, or maternal death.[1] Several of these may cause acute fetal anemia. Fetal anemia may be suspected from abnormal heart rate patterns. In the absence of the use of medications such as meperidine or anesthetics, sinusoidal patterns have been associated with fetal anemia, hypoxia, or asphyxia. In term or near term pregnancies the presence of a sinusoidal pattern after maternal trauma may be an indication for delivery. However, in the presence of prematurity further testing may be warranted to assess the need for delivery.

Recently, Fischer et al.[2] have reported a case of chronic fetomaternal hemorrhage managed with repeated fetal intravascular transfusions. The fetal anemia in our case was not due to fetomaternal hemorrhage as evidenced by the Kleihauer-Betke results. It may have been caused by a combination of hemorrhage from a ruptured vessel in the fetal side of the placenta and trauma-induced intracranial hemorrhage. This would explain the bloody amniotic fluid, the infarct in the fetal side of the placenta, and the neonatal brain findings.

This case demonstrates the possible adverse effects of blunt maternal abdominal trauma during pregnancy and the role that cordocentesis might play in the evaluation of abnormal fetal heart rate patterns. It also shows that delivery of a compromised fetus is not always the only management alternative. We were able to treat and resolve the fetal anemia with intravascular trans-

fusion and thus prolong this pregnancy. The unfortunate development of microcephaly secondary to the large parietooccipital brain lesion shows all too clearly that there are a number of unpredictable and unpreventable side effects of trauma during pregnancy.

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Infection and labor

VII. Microbial invasion of the amniotic cavity in spontaneous rupture of membranes at term

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OBJECTIVE: The purpose of this study was to determine the frequency, microbiologic characteristics, and clinical significance of microbial invasion of the amniotic cavity in women with premature rupture of membranes at term.
STUDY DESIGN: Amniocentesis was performed in 32 women with term premature rupture of membranes and amniotic fluid cultured for aerobic and anaerobic bacteria and Mycoplasmas.
RESULTS: The prevalence of positive amniotic fluid cultures was 34.3% (11/32). The most common isolates were *Ureaplasma urealyticum, Peptostreptococcus* sp., *Lactobacillus* sp., *Bacteroides fragilis,* and *Fusobacterium* sp. Clinical chorioamnionitis occurred only in one patient with a positive amniotic fluid culture. Her neonate had ophthalmitis. Three patients (9.4%) had endometritis. Among women who were delivered vaginally, those with a positive amniotic fluid culture had a significantly higher rate of endometritis than those with a negative culture (33% [3/9] vs 0% [0/20], respectively, $p = 0.023$).
CONCLUSIONS: These data indicate that microbial invasion of the amniotic cavity occurs in approximately one third of patients with preterm premature rupture of membranes. Microbial invasion of the amniotic cavity is a risk factor for endometritis in women with term premature rupture of membranes. (AM J OBSTET GYNECOL 1992;166:129-33.)

Key words: Rupture of membranes, amniotic fluid, Gram stain, limulus amebocyte lysate, chorioamnionitis, intraamniotic infection

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6/1/31254

Several studies have reported on the prevalence of microbial invasion of the amniotic cavity in preterm premature rupture of membranes.[1-7] However, there is no information available regarding the microbiologic state of the amniotic cavity during rupture of membranes at term. Previous studies addressing this issue have retrieved amniotic fluid with a transcervical intrauterine catheter from women in labor.[8] With this method, it is difficult to discern between positive cultures that are due to contamination of the fluid with cervicovaginal flora and true microbial invasion of the amniotic cavity. The purpose of this study was to de-

Table I. Clinical characteristic of study population according to amniotic fluid culture results

Characteristic	*Amniotic fluid culture results*	
	Positive (n = 11)	*Negative (n = 21)*
Maternal age (yr, mean ± SD)	25.4 ± 6.1	22.7 ± 5.9
Parity		
Primiparous	5	15
Multiparous	6	6
PROM-to-amniocentesis interval (hr)		
Median	4.5	9.0
Range	2-14	2-52
Amniocentesis-to-delivery interval (hr)		
Median	20.5	16.0
Range	5-33	3-41
Mode of delivery		
Vaginal	9	20
Cesarean section	2	1
Birth weight (gm, mean ± SD)	3478 ± 360	
Apgar score		
1 min	8.7 ± 0.4	8.9 ± 0.3
5 min	9.0 ± 0.3	9.0 ± 0.2

PROM, Premature rupture of membranes.

termine the prevalence and microbiologic features of microbial invasion of the amniotic cavity in women with term spontaneous rupture of membranes by transabdominal amniocentesis.

Material and methods

Patient population. A retrospective study was designed to identify women who had amniotic fluid retrieved transabdominally because of suspected preterm premature rupture of membranes but who subsequently were delivered of term neonates within 48 hours of amniocentesis. The diagnosis of rupture of membranes was made with sterile speculum examination and a combination of pooling, ferning, and Nitrazine tests. Amniocentesis for microbiologic studies is routinely offered in our institution to all patients admitted with suspected preterm premature rupture of membranes. The obstetric data base was searched to identify patients who had undergone amniocentesis but subsequently were delivered of neonates with birth weights >2500 gm, who were considered term infants by pediatric examination, and who had not undergone a digital examination before amniocentesis.

Microbiologic culture technique. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after collection. These conditions precluded air contact with the specimen. Plating occurred within 30 minutes of collection in all cases. Amniotic fluid was cultured for aerobic and anaerobic bacteria by methods previously described.[9] The samples were plated on blood agar, MacConkey's agar, Columbia colistin–nalidixic acid agar, and chocolate agar (36° C in 8% carbon dioxide) for aerobic culture and on blood agar, BBE (*Bacteroides,* bile-esculin agar), LKV (laked kanamycin, vancomycin agar), and Martin-Lewis agar (all prereduced) for anaerobic culture. Amniotic fluid was also inoculated in thioglycollate broth. Anaerobic cultures were grown in an anaerobic chamber in the Forum Scientific anaerobic system (model 1024; Division of Mallinckrodt, Marietta, Ohio). For *Mycoplasmas,* an aliquot of fluid (1 ml) was inoculated into transport media and frozen at −70° C until culture with techniques previously described.[10, 11] Gram stain examinations were performed with commercial reagents (crystal violet, saffranin, and Gram's iodine [Difco Laboratories, Detroit]) under standard conditions.[12] The limulus amebocyte lysate assay was performed according to techniques previously described.[13]

Patient management. Patients were managed according to the results of the Gram stain of amniotic fluid. The results of the limulus amebocyte lysate test were blinded. Patients with positive Gram stains received antibiotics (ampicillin and gentamicin), and labor was induced. Patients with negative Gram stains were managed expectantly.

Criteria for the diagnosis of chorioamnionitis and puerperal endometritis. Clinical chorioamnionitis was defined by the criteria proposed by Gibbs et al.[14] Puerperal endometritis was defined as a temperature elevation to 38° C or higher on two occasions (4 hours apart, excluding the day of delivery) with uterine tenderness, foul-smelling lochia and no other apparent source of infection. Routine neonatal cultures were not performed.

Statistical analysis. Comparisons between proportions were performed with either a χ^2 or Fisher's exact test. An unpaired Student *t* test was used to compare gestational age between groups. Computations were performed with the Epistat program (Epistat Services, Richardson, Tex.).

Results

Thirty-two patients met the criteria for inclusion into this study. All amniotic fluid samples were retrieved by transabdominal amniocentesis. The prevalence of positive amniotic fluid cultures was 34.3% (11/32). Table I displays the clinical characteristics of patients with positive and negative amniotic fluid cultures. There were no significant differences between the two groups in maternal age, parity, rupture of membranes–to–amniocentesis interval, amniocentesis-to-delivery interval, mode of delivery, mean birth weight, and Apgar scores at 1 and 5 minutes.

Table II. Results of microbiologic and diagnostic tests in women with ruptured membranes at term

Patient No.	*Gram stain*	*Limulus amebocyte lysate*	*Microorganism*	*Puerperal infection*
1	Negative	Positive	*Lactobacillus* sp.	No
2	Negative	Negative	*Ureaplasma urealyticum*	Endometritis
3	Negative	Positive	*Ureaplasma urealyticum*	No
4	Negative	Negative	*Ureaplasma urealyticum*	No
5	Negative	Negative	*Peptostreptococcus* *Ureaplasma urealyticum*	Endometritis
6	Negative	Negative	*Ureaplasma urealyticum*	No
7	Positive	Negative	*Streptococcus*, group G *Bacteroides fragilis* *Peptostreptococcus* sp. *Fusobacterium* sp. *Ureaplasma urealyticum*	Endometritis
8	Negative	Negative	*Fusobacterium* sp. *Ureaplasma urealyticum*	No
9	Positive	Positive	*Gardnerella vaginalis* *Ureaplasma urealyticum*	No
10	Negative	Positive	*Peptostreptococcus* sp. *Ureaplasma urealyticum*	No
11	Negative	Negative	*Lactobacillus* sp. *Bacteroides fragilis*	No

Table II illustrates the results of Gram stain, limulus amebocyte lysate, and microbiologic analysis of amniotic fluid of patients with positive amniotic fluid cultures. The most frequent isolates were *Ureaplasma urealyticum* (n = 10), *Peptostreptococcus* sp. (n = 4) and *Lactobacillus* sp. (n = 2). The prevalence of polymicrobial invasion of the amniotic cavity was 45.4% (5/11). The Gram stain examination of amniotic fluid was positive in 18.1% (2/11) of cases with a positive amniotic fluid culture. There were no false-positive Gram stains of amniotic fluid. The limulus amebocyte lysate test was positive in five cases (15.6% [5/32]), and in four of these patients the amniotic fluid was positive for microorganisms.

Clinical chorioamnionitis was diagnosed in one patient who had a positive amniotic fluid culture for *Gardnerella vaginalis* and *Ureaplasma urealyticum*. The overall prevalence of puerperal endometritis was 9.4% (3/32). Among women who were delivered vaginally, those with positive amniotic fluid cultures had a significantly higher rate of endometritis than did those with negative cultures (33% [3/9] vs 0% [0/20], respectively; p = 0.023, Fisher's exact test). One patient had both clinical chorioamnionitis and endometritis. Her neonate had ophthalmitis but no other clinical or laboratory signs of sepsis. However, neonatal cultures for *Mycoplasmas* were not performed.

Three patients were delivered by cesarean section, two with positive amniotic fluid cultures and one with a negative amniotic fluid culture. The indication for cesarean section was failure to progress in labor in all cases. Only the two neonates born to mothers with a positive Gram stain of amniotic fluid had blood cultures performed; they were negative for microorganisms. Neonatal septicemia was not diagnosed on clinical grounds in this series.

Comment

This is the first study to report the prevalence of microbial invasion of the amniotic cavity in women presenting with ruptured membranes at term. The results are unexpected, in that the prevalence of microbial invasion of the amniotic cavity at term is similar to that found in preterm premature rupture of membranes (34.3% [11/32] vs 28.5% [178/625], respectively).[1-7, 15] These findings imply that one third of patients with rupture of membranes have microbial invasion of the amniotic cavity, regardless of whether this complication occurs in the preterm or term gestation. A limitation of the current study is that amniocentesis was performed on all patients because they were thought to have preterm gestations. Although all newborns had weights of >2500 gm and were term by pediatric examination, our population may not represent the entire term population.

The most common microbial isolates in this study were *Ureaplasma urealyticum*, *Lactobacillus* sp., *Peptostreptococcus* sp., *Bacteroides fragilis*, and *Fusobacterium* sp. Most of these microorganisms are frequent constituents of normal cervicovaginal flora; therefore an ascending route is the most likely pathway for microbial invasion. Further studies including quantitative microbiologic analysis are required to establish whether there are differences in microbial inoculum size and type between women with term and preterm rupture of membranes.

What is the temporal relationship between microbial invasion of the amniotic cavity and rupture of mem-

branes? The traditional view has been that microorganisms gain access to the amniotic cavity after rupture of membranes. An alternative view is that microbial invasion of the amniotic cavity precedes the rupture of membranes. The following evidence supports this view. First, 13.6% of women with preterm labor and intact membranes have a positive amniotic fluid culture for microorganisms, and they are more likely to have spontaneous rupture of membranes that are women with a negative amniotic fluid culture.[12, 16-24] Second, incubation of fetal membranes with bacterial products or activated neutrophils reduces the bursting tension of fetal membranes in vitro.[25] Third, the prevalence of acute histologic chorioamnionitis is significantly higher in women with premature rupture of membranes than in women without premature rupture of membranes.[26] This effect is present even when the duration of rupture of membranes is <4 hours, implying that the inflammatory process occurred before rupture of membranes. Fourth, neonates born to women with premature rupture of membranes have increased levels of immunoglobulin M or A_1, suggesting that the fetal immune system had time to respond to a preexisting insult (i.e., infection) of some duration.[27] Fifth, experimental studies indicate that bacteria can cross intact fetal membranes in vitro.[28] Microbial invasion of the amniotic cavity may then lead to rupture of membranes. Collectively, this evidence suggests that microbial invasion of the amniotic cavity in cases with premature rupture of membranes may occur before rupture. In this study the interval between rupture of membranes and amniocentesis was not different in women with positive and negative amniotic fluid cultures. However, the number of observations is insufficient to exclude the possibility that microbial invasion of the amniotic cavity is a time-dependent phenomenon.

An important question is why microbial invasion of the amniotic cavity occurs in some women with rupture of membranes and not in others. Premature rupture of membranes is probably a heterogeneous condition caused by several factors[29]; intraamniotic infection is merely one of these factors. A positive amniotic fluid culture may reflect both the contribution of infection to the etiology of premature rupture of membranes and secondary microbial infection. Susceptibility to microbial invasion of the amniotic cavity after premature rupture of membranes may vary. Host factors, such as the antimicrobial properties of the amniotic fluid, may play a role in determining an individual's susceptibility.[30-38]

Our data indicate that microbial invasion of the amniotic cavity often occurs without demonstrable clinical signs of maternal or neonatal infection. Indeed, only one of 11 patients with positive amniotic fluid cultures had clinical chorioamnionitis. This clearly demonstrates that the currently used definition of clinical chorioamnionitis is an insensitive indicator of microbial invasion of the amniotic cavity. This observation is in keeping with those made in the context of preterm labor with intact membranes[24] and of term labor with intact membranes.[39]

Although microbial invasion of the amniotic cavity at term is not often accompanied by clinical signs of chorioamnionitis, this condition is not innocuous. All cases of puerperal endometritis occurred in women with positive cultures. Furthermore, the rate of endometritis after a vaginal delivery was greater in women with a positive amniotic fluid culture than in those with a negative culture. Since there were only three cesarean sections in this study, we cannot determine whether microbial invasion of the amniotic fluid also increases the risk of post–cesarean section endometritis. Previous studies, however, have indicated that rupture of membranes is an important risk factor for post–cesarean section endometritis. The possibility must be strongly considered that the basis for this association is the high rate of microbial invasion of the amniotic cavity in women with term premature rupture of membranes.

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Serum levels of macrophage colony-stimulating factor in patients with ovarian cancer undergoing second-look laparotomy

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OBJECTIVE: The purpose of this study was to evaluate the prognostic significance of macrophage colony-stimulating factor serum levels in patients with ovarian cancer undergoing second-look laparotomy.

STUDY DESIGN: The presurgical serum levels of macrophage colony-stimulating factor from 33 consecutive patients with ovarian cancer undergoing second-look laparotomy were determined and compared with those of 50 healthy control subjects. Mean differences in groups were evaluated with the Student *t* test.

RESULTS: Sixteen of 33 patients had a positive result at second look and a mean serum macrophage colony-stimulating factor level of 2.31 ± 1.45 ng/ml. Seventeen of 33 patients had a negative result at second look and a mean macrophage colony-stimulating factor level of 1.90 ± 0.86 ng/ml ($p > 0.05$). The mean macrophage colony-stimulating factor level in the control group was 1.20 ± 0.51 ng/ml. This was statistically lower than the mean level found in patients with a negative second-look result ($p < 0.05$).

CONCLUSION: Regardless of tumor status, serum macrophage colony-stimulating factor levels tend to be elevated at the time of second-look laparotomy. (AM J OBSTET GYNECOL 1992;166:134-7.)

Key words: Ovarian cancer, tumor marker, macrophage colony-stimulating factor, second laparotomy

Tumor cells produce growth factors, some of which may regulate the proliferation of cancer cells by autocrine and paracrine pathways.[1-7] The aberrant production of trophic factors by cancer cells is due to either gene amplification or genetic translocation, which may bring the growth factor genes under the control of different regulatory elements. Interestingly, among the different growth factors studied thus far, colony-stimulating activity has been detected in many nonhematologic tumors.[6, 7] Concomitant with the presence of colony-stimulating factor activity, higher levels of circulating leukocytes have been seen in some of these patients.[4, 5] These results, along with studies using murine experimental model systems wherein human tumors were transplanted, indicate constitutive production of cytokines by malignant cells.[7] Although the importance of these factors remains uncertain at the present time, their levels in serum may prove useful as markers to monitor primary tumors or to detect metastases.

Table I. Patient age

Group	*No.*	*Age (yr, mean ± SD)*
Control	50	41 ± 11
Second-look laparotomy	33	52 ± 14
Positive	16	55 ± 12
Negative	17	49 ± 16

Table II. Initial diagnosis of patients undergoing second-look laparotomy

Initial diagnosis	*No.*
Adenocarcinoma (serous or mucinous)	28
Malignant endometrioid tumor	1
Clear cell carcinoma	4
TOTAL	33

In a recent study it was reported that each of six ovarian cancer cell lines secrete macrophage colony-stimulating factor into the culture medium in the absence of any external stimuli.[8] Whereas lymphoid and myeloid cell lines failed to show any detectable amount

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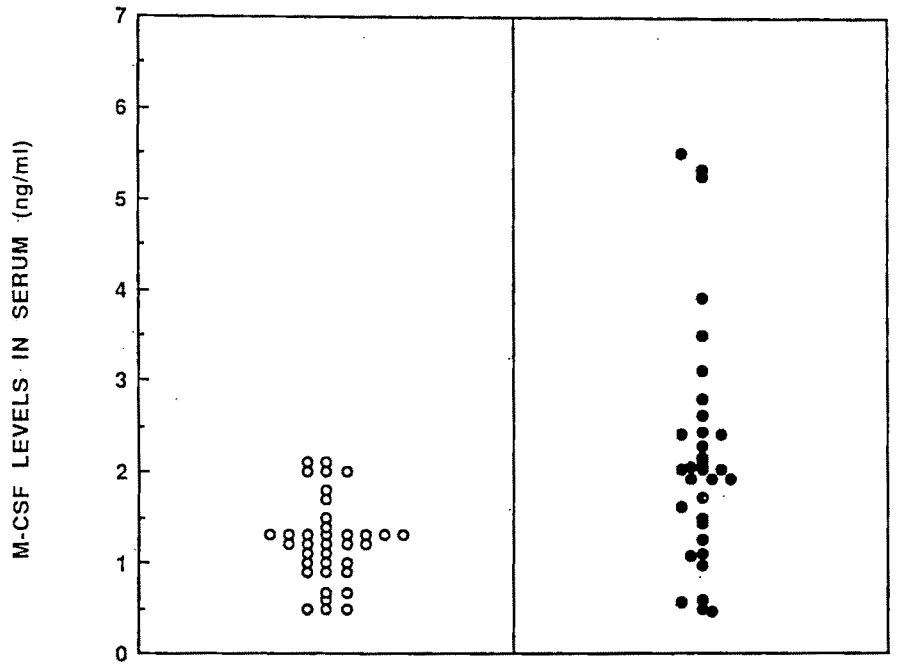


Fig. 1. Macrophage CSF levels in sera of normal control subjects *(open circles)* and patients with ovarian cancer before second-look laparotomy *(solid circles)*. Sera samples were analyzed in specific radioimmunoassay. Each value is mean of triplicate independent estimations from individual samples.

of macrophage CSF, a limited number of breast cancer cell lines produced the growth factor in significant quantities. Presence of the colony-stimulating factor was confirmed not only by immunologic (radioimmunoassay) and biologic assay (murine bone marrow cultures) but also by analysis of total cellular ribonucleic acid for macrophage CSF–related transcripts.[8] Kacinski et al.[9] have detected expression of macrophage CSF transcripts in ovarian carcinoma cells by in situ hybridization. Macrophage CSF is a dimeric glycoprotein of about 70 kd and belongs to a group of cytokines that regulate the ontogeny of blood cells. Macrophage CSF selectively stimulates proliferation and differentiation of the mononuclear phagocytic lineage and mature macrophages.[10, 11] Recently, macrophage CSF serum levels were reported to be elevated in patients with epithelial ovarian cancer.[9] It has been suggested that macrophage CSF may be useful as a potential tumor marker. The purpose of this study is to evaluate the prognostic significance of macrophage CSF levels in ovarian cancer patients undergoing second-look laparotomy.

Material and methods

Serum samples from 33 consecutive patients undergoing second-look laparotomy for epithelial ovarian cancer were retrieved from the serum bank of the University of Minnesota Women's Cancer Center. The sera, which had been drawn just before surgery, were obtained between Dec. 1, 1985, and June 1, 1988, and stored at −20° C until analysis.

Macrophage CSF levels in these samples were determined in the following way. A polyclonal antiserum against human recombinant macrophage CSF (provided by the Genetics Institute, Cambridge, Mass.) was generated in rabbits by hyperimmunization. The antiserum used in these studies has been well characterized and does not cross react with other growth factors or cytokines. Details of the assay conditions are published elsewhere.[8] This assay system can detect concentrations as low as 500 pg/ml. To minimize interassay variations, aliquots of individual sera samples from five normal subjects were used as internal controls. Macrophage CSF serum levels were determined in a similar manner in 50 healthy female controls.

All samples were also assayed for the presence of tumor marker CA 125 with an immunoradiometric method.[12] CA 125 levels were expressed in relative units per milliliter. Mean differences in groups were evaluated with the Student t test. A p value <0.05 was considered significant.

Results

No clinical evidence of infection was noted at the time of obtaining the serum. Mean ages of the study groups are given in Table I. A summary of initial pathologic diagnoses is shown in Table II. Macrophage CSF levels as determined by radioimmunoassay showed a wide

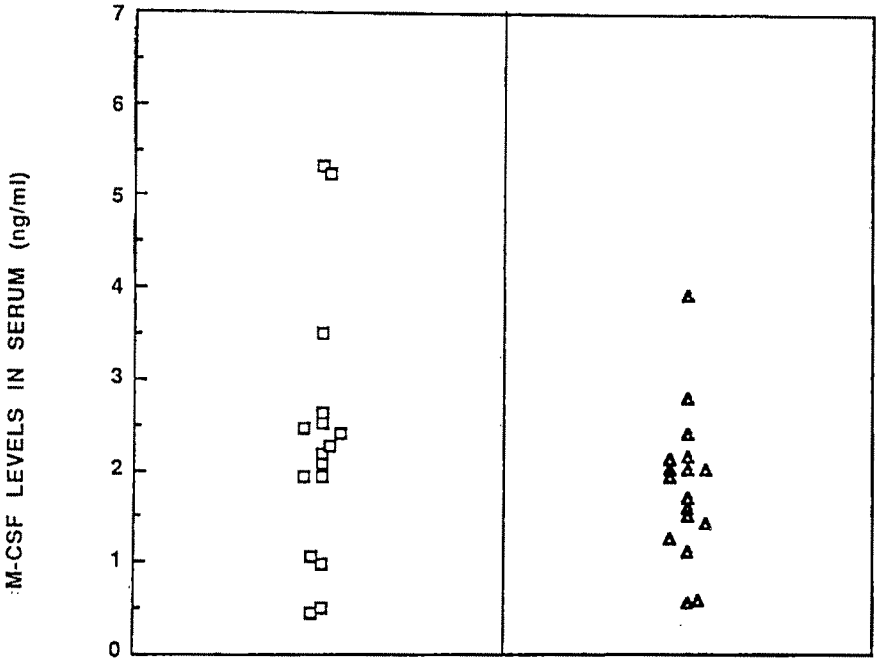


Fig. 2. Macrophage CSF levels in sera of patients with ovarian cancer. *Open squares,* Abnormal pathologic findings at second-look laparotomy; *open triangles,* normal pathologic findings at second-look laparotomy.

range between nondetectable levels and 5.3 ng/ml in one patient. The distribution of individual samples is shown in scatter plots (Figs. 1 and 2). Sixteen patients with positive pathologic studies at the time of second look had a mean serum macrophage CSF level of 2.31 ± 1.45 ng/ml. The mean level in the 17 patients with negative findings was 1.90 ± 0.86 ng/ml. The difference in these levels is not statistically significant ($p > 0.05$).

The mean macrophage CSF level in the control group was 1.20 ± 0.51 ng/ml. This is statistically lower than that found in patients with a negative second look ($p < 0.005$). Subsequently, macrophge CSF levels were compared with those of another tumor marker, CA 125. In all but five of the 33 patients, CA 125 levels were negative (<35 U/ml). Two of these five had a macrophage CSF level <3.62 ng/ml (mean ± SD 1.90 + 2) and a negative second look, one had a positive second look and a macrophage CSF level >3.62 ng/ml, and two had a positive second look and a macrophage CSF level <3.62 ng/ml (0.98 and 3.50 ng/ml). The variations seen in the levels of macrophage CSF might be attributed to tumor load. However, in the current study the sample size was too small to evaluate a possible correlation at second-look laparotomy.

Comment

Under normal conditions, macrophage CSF is produced constitutively by a variety of cells, including fibroblasts, endothelial and epithelial cells, and placental tissue.[8, 13, 14] Certain functionally differentiated cells such as monocytes do not secrete detectable quantities of macrophage CSF unless stimulated with tumor-promoting reagents (e.g., phorbol myristate acetate) or with bacterial lipopolysaccharides.[15-17] Normal serum will contain some basal amount of colony-stimulating factor. A recent report by Shaddle et al.[18] indicated that normal serum contained a mean level of 1.5 ng/ml of macrophage CSF, which is comparable to the levels observed in our study. The steady-state level of macrophage CSF in serum is governed by the rates of production and clearance. In normal individuals these two parameters are at equilibrium. Although the clearance rate and compartments involved in the clearance process have not been completely resolved for macrophage CSF, alveolar macrophages and Kupffer cells in the liver contribute to the removal of macrophage CSF from the circulation.[19] Macrophage CSF has a very short half-life, and injection of radioiodinated macrophage CSF is quickly cleared from circulation within 15 minutes. It has been noted that elevated macrophage CSF levels are associated with renal dysfunction.

In an earlier study Kacinski et al.[9] have shown that macrophage CSF levels in patients with ovarian cancer were significantly elevated. The increase had a positive correlation with CA 125 levels and showed a marked decrease after tumor debulking. These observations suggested that macrophage CSF levels could be used as an adjunct diagnostic parameter in ovarian cancer.

In this study we investigated the possible predictive value of macrophage CSF levels in patients undergoing second-look operation who had low CA 125 levels. All but one of the 33 patients studied received cisplatin before second look. One patient had a renal transplant before ovarian cancer developed. Although significant nephrotoxicity was documented in only two patients, subtle renal toxicity may also account for the elevated macrophage CSF levels found in patients with no evidence of disease at the time of second look. Renal function was not evaluated in the control group.

Macrophage CSF might be elevated simply because the patients in the study had been on a regimen of chronic leukocyte-suppressing chemotherapy. All patients, however, had normal white blood cell counts at the time of second-look surgery. Further study is warranted to see whether all patients undergoing chemotherapy have an elevated macrophage CSF level.

We conclude that, regardless of tumor status, macrophage CSF levels tend to be elevated at the time of second-look laparotomy. Further work is currently under way to determine if macrophage CSF levels may predict eventual recurrences in those patients with negative second-look laparotomy.

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Population differences of fetal biophysical and behavioral characteristics

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Population differences in nonstress test reactivity have been reported with a threefold increase in the likelihood of nonreactive nonstress tests observed in black fetuses as compared with white fetuses. We analyzed fetal behavioral states and fetal heart rates in 14 black and 15 white fetuses at term to explain this observed difference in nonstress test reactivity. Two-hour Doppler and real-time ultrasonographic examination of each patient revealed no differences in percent time spent in the four behavioral states between the two populations. A 9.5 beats/min difference between black and white fetuses was found. The higher baseline heart rate of the black fetuses persisted in each behavioral state and may affect nonstress test reactivity because of rate-dependent decreases in short-term variability and rate-dependent limitations of maximal acceleration amplitude. (AM J OBSTET GYNECOL 1992;166:138-42.)

Key words: Heart rate, fetal, population differences, fetal surveillance

The nonstress test (NST) is an antepartum method of assuring fetal well-being in pregnancy. A "reactive" test is reassuring, indicating fetal heart rate (FHR) accelerations coincidental with fetal movement. It has recently been reported that maternal race may influence NST results, with black women noted to have nonreactive or nonreassuring tests three times more often than white women.[1] This large study determined the number and percentage of nonreactive NST results by demographic and behavioral characteristics. Fourteen percent (180/1263) of the blacks studied had nonreactive NSTs as compared with 4.3% of the whites studied (28/658). After controlling for gestational age, maternal medical complications, and behavioral characteristics such as substance abuse, this difference persisted. In a prospective study designed to evaluate auscultated FHR accelerations in conjunction with vibroacoustic stimulation, blacks were again significantly more likely to have a nonreactive NST.[2] Inherent racial variation in mean baseline heart rate could explain this observed population difference. The increased "nonreactivity" of the heart rate in black fetuses could also be attributed to racial variation in fetal behaviors such as movement and breathing. Fetal behavioral states have been described according to the parameters of FHR pattern, eye movements, breathing, and gross body movements.[3] The premise that black fetuses spent more time in states associated with "nonreactivity" (1F and 3F) than white fetuses could explain the increased incidence of nonreactive NSTs. The current study was undertaken to ascertain if this difference in reactivity could be attributed to differences in time spent by black and white fetuses in the four fetal behavioral states.

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Methods

Patients. Informed consent was obtained from healthy, nonsmoking pregnant women who were between 38 and 41 weeks' gestation with uncomplicated pregnancies. The protocol was approved by the institutional review board at our institution. Women were recruited from the low-risk obstetric clinic, as well as from university-based private practices. The sample comprised 15 white and 14 black women.

The women were studied in our fetal testing unit after eating lunch. They were placed in a left lateral supine position and were monitored for 2 hours on the Toitu fetal actocardiograph model MT320. This monitor records both FHR and fetal movement; its reliability has been previously reported.[4] An Aloka real-time ultrasonographic transducer was positioned over the fetal head to observe fetal lens movement. This was simultaneously recorded on the heart rate strip with an event marker (Fig. 1).

The 29 women were monitored for an average of 120 minutes, with a range of 99 to 155 minutes. The FHR was determined for each fetus by visual inspection of the monitor strip (by M.J.J.). Changes in baseline of at least 3 minutes' duration were recorded and an average baseline for each subject was computed. A second observer (C.G.) reread the baseline on 10 randomly

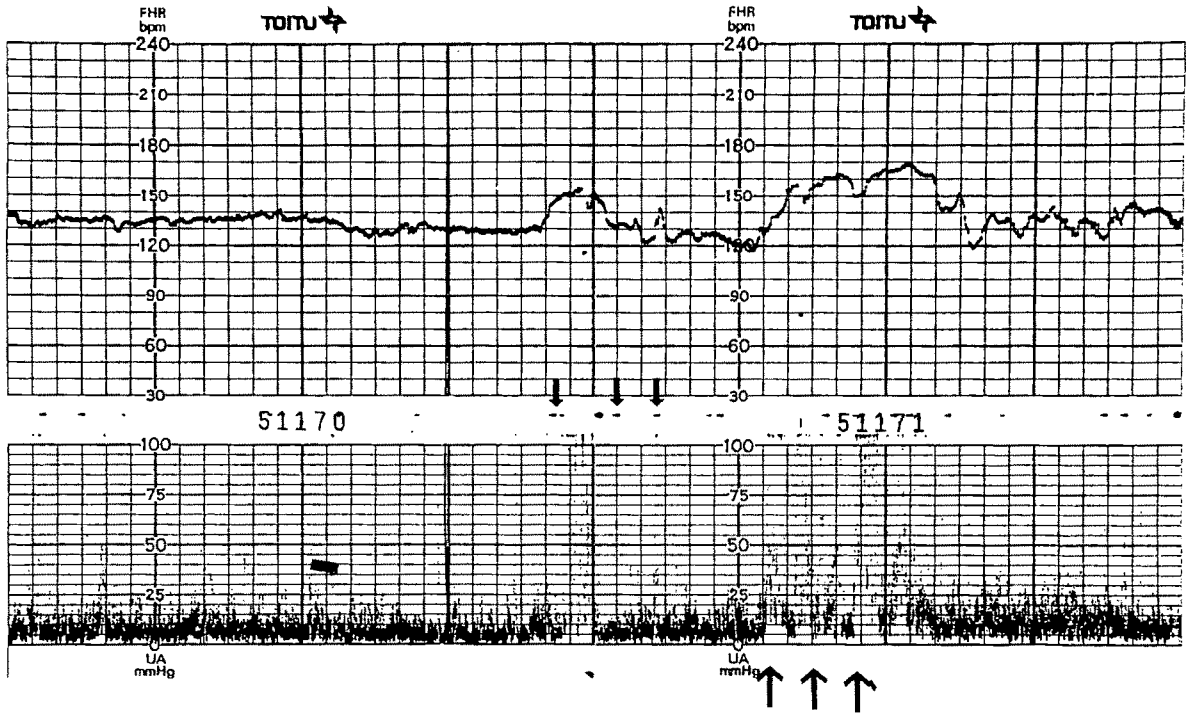


Fig. 1. Representative FHR recording. *Top line* is external FHR recording, with acceleration concomitant with fetal movement (depicted by ↑ on actograph). Fetal eye movements are recorded by event marker (↓).

selected strips. Pearson's correlation coefficient of these two readings was excellent at 0.984. The monitor strips were then evaluated for fetal state. FHRs were classified into patterns A, B, C, and D according to a previously described method.[5] Fetal body movements as depicted by the actograph were deemed present or absent. Fetal eye movements were likewise noted as present or absent. The three parameters of FHR pattern, body movement, and eye movements were then combined and fetal state assigned in a modification of Prechtl's criteria by a single investigator (T.J.).[5] Consensus on FHR patterns and states was provided by a second investigator (M.J.J.).

Percent time in each fetal state and mean baseline during each state were then computed for each fetus. Statistical significance was determined for demographic and clinical characteristics by means of χ^2, Fisher's exact test, and independent t tests as appropriate. Independent t tests were also used to evaluate mean percent time in the fetal behavior states and mean FHRs. The 0.05 confidence level was used to detect statistical differences throughout the analysis. The SYSTAT statistical package was used to analyze the data.

Results

Fetal outcome was good in all patients. The mean birth weight in the black fetuses was 3414 gm, which was not statistically different from the mean birth weight in the white fetuses, which was 3689 gm ($t = -1.513, p = 0.142$). There were no 5-minute Apgar scores <8.

Table I. Characteristics of study groups

	Black patients	*White patients*
Status		
Clinic	14	2*
Private	0	13*
Parity		
Primiparous	9	8
Multiparous	5	7
Mean maternal age (yr)	20.4	30.7*
Mean test gestational age (wk)	38.7	38.6

*$p < 0.05$.

The characteristics of the study groups are enumerated in Table I. The two racial groups were significantly different with regard to clinic status and maternal age. Parity was not significantly different. There were nine primiparous women in the black group and eight in the white group.

No significant differences in fetal state were found between the two groups (Table II). The black fetuses were in a "quiet-sleep" state 1F 28% of the monitored time; the white fetuses spent 22% of the monitored time in 1F. The FHR pattern in 1F shows minimal

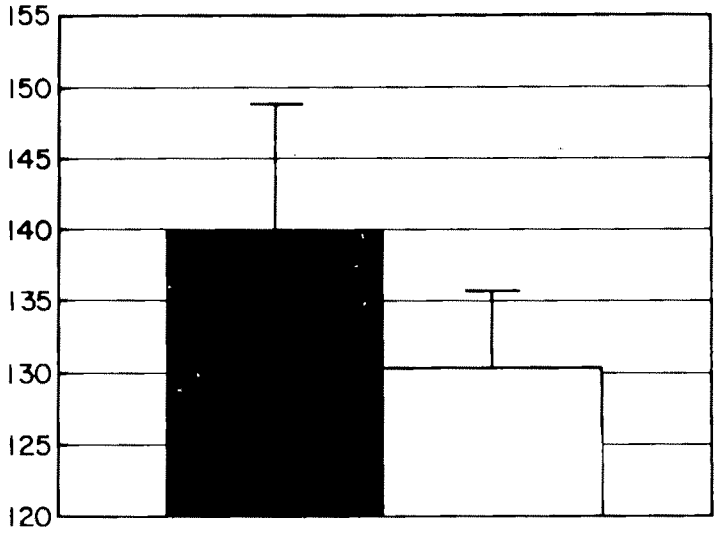


Fig. 2. Mean baseline FHR. ■, Black patients; □, white patients; $p = 0.003$.

Table II. Percent time in states 1F to 4F*

Behavioral state	*Time (%, mean ± SD)*	*Statistical significance*
1F		
Black patients	27.9 ± 18.7	$p > 0.05$
White patients	21.8 ± 17.1	
2F		
Black patients	51.8 ± 21.0	$p > 0.05$
White patients	61.7 ± 26.1	
4F		
Black patients	15.8 ± 26.5	$p > 0.05$
White patients	11.2 ± 19.1	

*Insufficient data for state 3F.

variability and is "nonreactive," according to the usual definition of two accelerations of 15 beats/min and 15 seconds' duration in a 20-minute window. Both groups of fetuses spent the majority of the monitored time in the "active-asleep" state 2F (blacks 52%, whites 62%), again with no statistical racial difference noted. The fetal heart rate pattern in 2F is typically "reactive" with frequent heart rate accelerations coincidental with fetal body movements. The fetuses spent minimal time in state 3F, in agreement with other authors[7] (blacks 4%, whites 5%). The"active-awake" state 4F occurred in 16% of the monitored time in the black fetuses as compared with 11% of the monitored time in the white fetuses, a difference that was not statistically significant. Therefore black and white fetuses spent equal times in the four behavioral states; when the "reactive" states (2F and 4F) were combined and compared with the "nonreactive" states (1F and 3F), there was again no significant difference. When the overall mean FHRs were analyzed, a significant difference in baseline was found between the two groups (Fig. 2). Black fetuses had a mean baseline of 139.9 beats/min, which was 9.5 beats/min faster than that of white fetuses (130.4 beats/min). The FHR baseline was examined for each behavioral state for blacks and whites (Fig. 3). The higher baseline in black fetuses persisted in each state, reaching a difference of 13 beats/min in 4F.

The significant difference in mean FHR was evaluated after fetal sex was controlled for. No differences by race were observed; however, it should be noted that the sample sizes were small (Table III). Similarly, newborn birth weight did not correlate with baseline FHR ($r = -0.05$).

Comment

Multiple maternal behavioral variables and medical complications have been shown to directly affect antepartum fetal assessment test results. Variability in

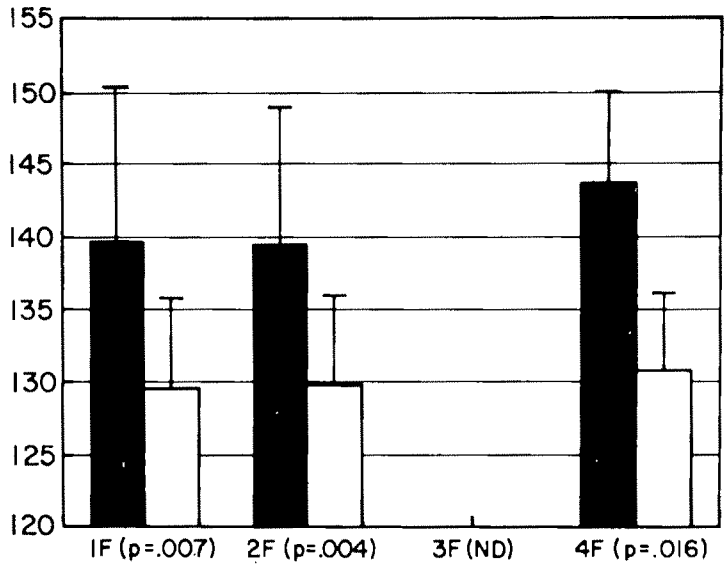


Fig. 3. Mean baseline FHR for states 1F to 4F. ■, Black patients; □, white patients. *ND*, Insufficient data.

Table III. Gender differences in mean FHR by race

	Black patients			*White patients*		
Sex of fetuses	*No.*	*FHR (beats/min, mean ± SD)*	*Statistical significance*	*No.*	*FHR (beats/min, mean ± SD)*	*Statistical significance*
Male	11	140.0 ± 10.1	$p > 0.05$	6	128.3 ± 4.9	$p > 0.05$
Female	3	139.7 ± 4.5		9	133.5 ± 4.7	

FHR reactivity, as well as various biophysical parameters, is influenced by gestational age, length of testing, and maternal ingestion of drugs, alcohol, cigarettes, and glucose.[6-9] The parameter of race, or ethnicity, has not been explored in this area although the literature is rich with heart rate and blood pressure data in children and adults of different races.[10, 11]

Our initial premise that the significant difference in NST reactivity between the black and white groups might be attributed to behavioral state differences could not be validated when 2-hour evaluations of 14 black and 15 white fetuses were undertaken. We did, however, find a significant difference in mean baseline heart rate, which has not been well described in research of the fetus. Schacter et al.[12] have consistently demonstrated a higher heart rate in black newborns as compared with white newborns. Seventy-four white and 67 black healthy term newborns to women of low socioeconomic status had heart rate measurements taken for three ½-hour periods of quiet, non-rapid-eye-movement sleep. Both the slowest heart rate and the mean heart rate were significantly faster for black newborns than for white newborns, with this difference averaging 8 beats/min. Schacter et al.[13] further evaluated the socioeconomic status in black and white newborns and verified that this heart rate difference persisted irrespective of socioeconomic status.

It is easily discerned that our two study groups differed by socioeconomic status, as well as by race. The black patients were recruited from the obstetric clinic. Nonsmoking white patients were difficult to recruit from this clinic; therefore the majority of the white patients were from private practices. In recognition of these differences, we refrained from attributing the observed heart rate difference to race alone. Population differences in our study in maternal anxiety or unrecognized drug use could contribute to the differences found.

Our observation of heart rate differences in different populations poses many questions. Inherent population variations in birth weight have been determined.[14] Earlier physical maturity of black newborns compared with white newborns of similar gestational ages has been described.[15] Racial differences in fetal behaviors are only now being explored. Further investigation into this area should include the Hispanic population, especially since much of the original fetal assessment data comes from this ethnic group. Patrick et al.[16] have discussed the association between maternal and fetal heart rates; we did not evaluate this association in our study.

The clinical significance of this heart rate difference is obvious when one considers its potential effect on maximal acceleration amplitude. It has been demonstrated that fewer accelerations of 15 beats/min are mounted when the FHR is ≥140 beats/min.[17] Eight of our 14 black subjects had a mean FHR ≥140 beats/min in comparison with 2 of 15 white subjects who had a mean FHR ≥140 beats/min. Therefore race may be an important variable when the sensitivity and specificity of the NST, as well as other antepartum tests, are ascertained.

In this analysis black and white fetuses spent equal time in the four fetal behavioral states when studied for 2 hours in the afternoon. A difference of 9.5 beats/min in mean baseline heart rate was found between the two populations, a finding well documented in the neonatal literature but previously underrecognized in the obstetric literature. Population differences and fetal physiologic variables need to be further studied to augment our current knowledge of antepartum fetal assessment and testing.

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May-Hegglin anomaly: A rare case of maternal thrombocytopenia in pregnancy

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The May-Hegglin anomaly, a rare cause of thrombocytopenia, is an autosomal dominant disorder that may have adverse maternal and fetal consequences. We present herein a case of May-Hegglin anomaly in pregnancy. The characteristic features of this anomaly, clinical presentation, and management options are discussed. (AM J OBSTET GYNECOL 1992;166:143-4.)

Key words: May-Hegglin anomaly

We describe a case of May-Hegglin anomaly in pregnancy.

Case report

A 28-year-old, white woman, gravida 4, para 0-0-3-0, with spontaneous rupture of the membranes at >39 weeks, was transferred from an outlying hospital to Temple University Hospital for tertiary care. The patient had been diagnosed with the May-Hegglin anomaly several years earlier. The patient had bleeding gums 1 week before admission but was otherwise asymptomatic. Physical and ultrasonographic examination revealed a nonfrank breech presentation of the fetus. The maternal platelet count at the time of admission was 34,000/mm³. Peripheral blood smear was characteristic of the May-Hegglin anomaly, showing giant platelets and cresent-shaped inclusions in the cytoplasm of neutrophils and monocytes. In view of fetal presentation and the 50% risk of inheritance of this disorder, a primary low-transverse cesarean section was performed. She received 12 U of platelets before surgery, and the preoperative platelet count was 90,000/mm³. A viable female infant weighing 7 pounds 3 ounces was delivered with Apgar scores of 6 at 1 minute and 9 at 5 minutes. The patient required no additional platelet transfusions. Repeat platelet count before discharge was 117,000/mm³. The patient's postoperative course was uncomplicated, and she was discharged on the fourth postoperative day. The baby's platelet count was normal and did not show any characteristics of the May-Hegglin anomaly.

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Comment

The May-Hegglin anomaly is a rare hereditary platelet disorder transmitted in an autosomal dominant pattern. The disorder was first described in 1909 by May[1] when he noted the giant platelets and sky-blue, cresent-shaped inclusions in the cytoplasm of neutrophils, eosinophils, and monocytes. In 1945, Hegglin[2] observed thrombocytopenia and bleeding diathesis in three family members. The platelets in May-Hegglin anomaly are large, measuring up to 15 μm in diameter. They are elliptic, cigar-shaped, or rounded. Thrombocytopenia is usually mild, but platelet count may be as low as 10,000/mm³. The megakaryocyte level in bone marrow is normal, and it has been suggested that the basic defect may be impaired megakaryocyte fragmentation.[3] The inclusion bodies, measuring 1 to 2 μm in diameter, are best observed in neutrophils. Usually only one inclusion per cell is found, and on electron microscopy and histochemical staining, inclusions have been found to contain ribonucleic acid.

A computer-assisted search showed only four cases

Table I. Outcome of five cases of May-Hegglin anomaly in pregnancy

No.	Series	Platelet count (No./mm³)	Delivery route	Outcome	
				Maternal	Neonate
1	Landy et al.[4]	22,000	Cesarean section—placenta previa	Good	May-Hegglin anomaly
2	Duff and Jackson[5]	32,000	Cesarean section—May-Hegglin anomaly	Good	May-Hegglin anomaly
3	Gausis et al.[6]	50,000	Vaginal delivery	Good	Stillbirth
4	Kotelko[7]	24,000	Cesarean section—failure to descend	Good	May-Hegglin anomaly
5	Chatwani et al. (present case)	34,000	Cesarean section—nonfrank breech	Good	Normal

of this anomaly in pregnancy (Table I). Maternal diagnosis of this disease in pregnancy is established by characteristic peripheral smear and by excluding other causes of thrombocytopenia. Fetal diagnosis can be done in the same manner by periumbilical blood sampling. The obstetric management involves prevention of hemorrhage in both mother and fetus. At the time of delivery maternal hemorrhage can be avoided by transfusion of platelets as needed. Prenatal diagnosis will dictate route of delivery. In this case fetal diagnosis was not done because of the fetal presentation and because the information would not change the course of management. Failure to clearly assess fetal involvement may result in adverse fetal consequences, such as intracranial hemorrhage, during parturition. This case report simply documents a rare cause of maternal thrombocytopenia, which may have fatal consequences if such a diagnosis is not entertained and excluded.

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Oral magnesium and the prevention of preterm labor in a high-risk group of patients

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OBJECTIVE: The null hypothesis of this study is that treatment with oral magnesium gluconate 1 gm four times daily will not decrease the rate of preterm labor and delivery in a high-risk group of pregnant women.
STUDY DESIGN: Fifty-four women at risk for preterm delivery were selected randomly to receive magnesium gluconate 1 gm orally four times daily or placebo. These women were monitored prospectively for signs and symptoms of preterm labor. A subgroup of 31 women also received a home uterine activity monitor. The serum magnesium level was measured initially and again 2 weeks after study enrollment. The data were analyzed with Fisher's exact test and analysis of variance.
RESULTS: Preterm labor developed in 15 women in the placebo group and in 16 women in the magnesium group. There were no differences in birth weight or gestational age at delivery. The mean increase in serum magnesium level while the patients were taking magnesium gluconate was 0.10 mg/dl (p = not significant). Five women did not have an increase in serum magnesium level and preterm labor developed in all of them.
CONCLUSION: Magnesium gluconate in a dose of 1 gm four times daily is not effective for preventing preterm delivery in a high-risk group of patients. (Am J Obstet Gynecol 1992;166:144-7.)

Key words: Magnesium, preterm labor, tocolysis

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Preterm birth occurs in approximately 9% of all deliveries and is accompanied by high rates of perinatal morbidity and mortality.[1] Factors that put women at risk for early delivery can be identified.[2] Monitoring women more extensively for preterm labor results in a reduction in preterm births.[3]

The use of oral magnesium gluconate for tocolysis

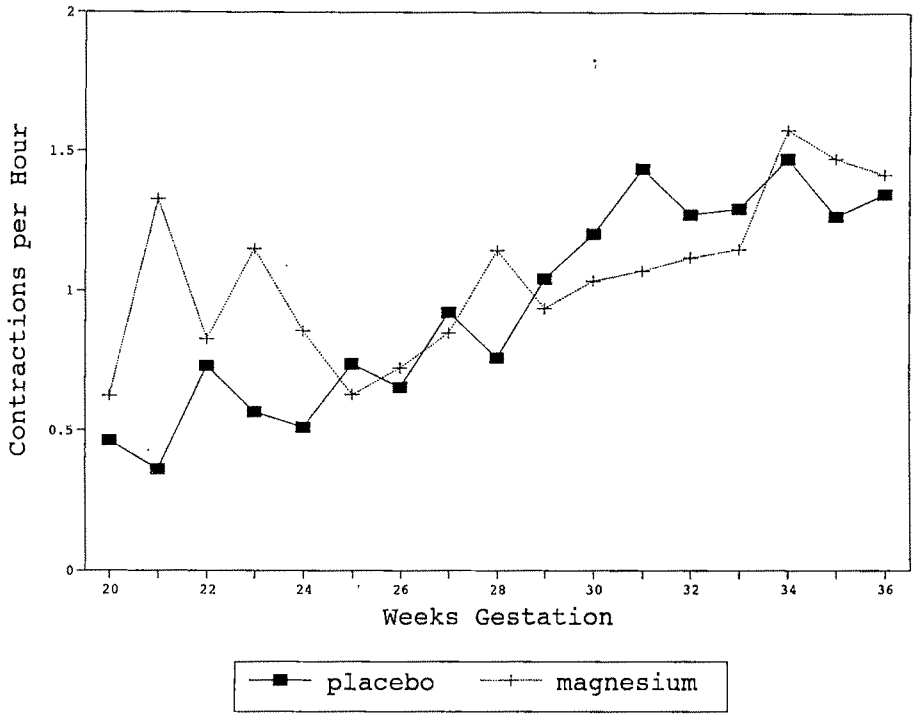


Fig. 1. Baseline uterine activity.

in ambulatory patients with preterm labor has been reported.[4] Magnesium is well tolerated with only minor gastrointestinal effects and is inexpensive compared with other oral tocolytic drugs. Finally, women who have low serum magnesium levels have a greater risk of preterm labor.[5] In uncontrolled studies supplementation of this ion has suggested a reduction in the incidence of early delivery.[6]

The purpose of this prospective study was to assess whether treatment with oral magnesium reduces the incidence of preterm labor and delivery in women at high risk for preterm birth.

Patients and methods

Fifty-four women with risk factors for preterm delivery were enrolled in the study, which was approved by the local institutional review board. After giving informed consent, the patients were randomly selected to receive either two 500 mg magnesium gluconate tablets four times daily or two placebo tablets four times daily. All tablets had the same physical appearance. Both groups were monitored prospectively in a high-risk clinic. The health care providers were blind as to which treatment each patient received. Patients were educated as to the signs and symptoms of preterm labor. A cervical examination was performed every 2 weeks. Preterm labor was diagnosed by the presence of regular, persistent uterine contractions with a change in cervical dilatation.

If preterm labor occurred, the study medication was discontinued and the patient was treated with standard tocolytic therapy (parenteral magnesium sulfate). Patients who completed 37 weeks' gestation returned to routine obstetric care. The gestational age at delivery, birth weight, and episodes of preterm labor were recorded.

After the study had begun, 31 consecutive patients (16 magnesium, 15 placebo) received home uterine ambulatory monitoring for 1 hour twice daily. The uterine activity data were transferred via telephone once daily to a perinatal nurse, who recorded the data and each patient's symptoms in a manner previously described.[7] The weekly uterine contraction rate was determined as an average of the uterine activity recorded during the monitored periods (2 hours daily, 7 days each week). Uterine activity increased markedly in the few days before the onset of preterm labor. To obtain a baseline contraction rate, we excluded the contraction data in the week before the onset of preterm labor.

The data were evaluated with the Fisher exact test and analysis of variance, as appropriate. A p value <0.05 was considered to be significant.

Results

Seven of the 54 women in the study were excluded from analysis because they moved from the area (4 women), experienced an early pregnancy loss (2), or violated the study protocol (1). Of the remaining 47

Table I. Risk factors for preterm labor

Risk factor	*Magnesium group*	*Placebo group*
Prior preterm delivery	17	19
Multiple gestation	1	5
Cerclage procedure	1	0
Uterine activity	3	0
Leiomyomas	0	1
TOTAL	22	25

patients, 25 were assigned to the placebo group and 22 were assigned to the magnesium group. The average maternal age was 26.5 years in the placebo group and 24.2 years in the magnesium group. The mean parity was 2.4 in the placebo group and 1.7 in the magnesium group. Gestational age at entry into the study was not different, with 23.4 and 23.7 weeks in the placebo and magnesium groups, respectively. There were no significant differences in the entry characteristics. Risk factors for preterm delivery were also similar in each group (Table I).

Fifteen women in the placebo group had preterm labor, compared with 16 in the magnesium group. The average gestational age (mean ± SD) at delivery was 35.6 ± 3.7 weeks for the placebo group and 35.6 ± 2.8 weeks for the magnesium group. Mean birth weights were similar: 2612 ± 828 gm in the placebo group and 2479 ± 684 gm in the magnesium group. Only two infants scored <7 on the Apgar scale at 5 minutes. Both infants were delivered of mothers in the placebo group.

No major side effects were reported among the 22 women in the magnesium group. Serum magnesium levels were obtained for 36 patients (21 placebo group, 15 magnesium group) initially and 2 weeks after they entered the study. The initial serum magnesium level was 1.68 ± 0.25 mg/dl for the placebo group and 1.64 ± 0.21 mg/dl for the magnesium group. Women taking magnesium gluconate responded with a rise in serum magnesium level of 0.10 ± 0.21 mg/dl from the initial measurement ($p < 0.09$, paired t test). The serum magnesium level fell by 0.01 ± 0.23 mg/dl in those not receiving magnesium.

The serial magnesium values obtained in 15 patients taking magnesium gluconate showed 4 of 10 patients with increased serum magnesium levels having preterm labor. It is interesting that all five women whose serum magnesium levels did not increase had preterm labor ($p < 0.15$).

Uterine activity data are presented in Fig. 1. The mean hourly uterine activity increased with gestational age in both groups. The data were analyzed with a two-factor analysis of variance (treatment, time), with one factor (time) repeated. The difference between the magnesium and placebo groups was not statistically significant ($p < 0.8$).

Comment

Magnesium exerts an effect on uterine contractility, presumably by its competition with intracellular calcium. In 1966 Rusu et al.[5] reported a decline in serum magnesium level with advancing gestation and a further fall before the onset of labor. They suggested a role for maternal administration of oral magnesium in the prevention of preterm birth. Later a retrospective study by Conradt et al.[6] established a decreased preterm delivery rate, from 11% to 4.9%, with the addition of oral magnesium to a standard treatment of cerclage and prophylactic β-sympathomimetic drugs. More recently in a prospective study Spatling and Spatling[8] were able to reduce the incidence of preterm labor from 9% to 4.3% in women treated with magnesium aspartate compared with women receiving placebos.

These initial results were encouraging. In other studies magnesium was found to be inexpensive and well tolerated when given to patients in preterm labor.[4] Many pregnant women, particularly women of lower socioeconomic status,[9] do not have adequate magnesium in their diets. This group of women has not always benefited from intervention with current risk-scoring systems to prevent preterm delivery.[10] A study to evaluate magnesium supplements for women at risk for preterm delivery would therefore be reasonable.

In our study prophylactic magnesium had no effect on the duration of pregnancy or infant birth weight. The study dosage of 216 mg of elemental magnesium was lower than the dosage used by Spatling and Spatling.[8] In our institution's earlier report of oral magnesium tocolysis, magnesium gluconate was given six times daily.[11] The four times per day regimen (30% lower than in the previous study) was designed to increase patient compliance. Whereas the increase in serum magnesium levels achieved statistical significance in the previous study, there was not a statistically significant rise in serum magnesium levels with the lower magnesium dosage. Sibai et al.[12] have also reported the use of prophylactic magnesium. Their study included primigravid women who were not at risk for preterm delivery. Although the focus of the study of Sibai et al. was to evaluate the effect of oral magnesium on pregnancy-induced hypertension, the incidence of preterm birth did not decrease in the magnesium-treated group.

There may be a subgroup of women who could benefit from a program of magnesium prophylaxis. The five women in this study whose serum magnesium levels did not increase may represent such a group. These women may have had relative magnesium deficiency despite normal initial serum magnesium levels. Palla et al.[13] described a magnesium load test in which pregnant women were given intravenous magnesium and the urinary excretion of this cation was measured. Retention of >20% of an intravenous magnesium load was consistent with magnesium deficiency. In our study, the

group of women with decreased serum magnesium levels may have been magnesium deficient and would perhaps benefit from a higher dosage of oral magnesium. A study with a larger number of patients is needed to investigate this aspect of magnesium replacement.

Although there was not a difference in gestational age at delivery or in birth weight in the two groups, it might be expected that the lower dose of magnesium would decrease uterine activity. In the 31 patients evaluated via home uterine activity monitoring the baseline hourly contraction rate increased with gestational age, as has been described in other studies.[14] There was, however, no difference in uterine activity between the placebo and treatment groups.

Oral magnesium gluconate did not change the incidence of preterm labor or infants with low birth weight in this high-risk group of women. Uterine activity was also not affected by this dosage of magnesium. Women with risk factors whose serum magnesium levels did not increase while on magnesium therapy may be at increased risk for preterm delivery.

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Pelvic abscess complicating transcervical embryo transfer

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A severe pelvic infection resulting in a tuboovarian abscess after transcervical embryo transfer is reported. The case is unique in that the recipient was an agonadal woman who had not undergone prior transvaginal aspiration. Although rare, pelvic infection after embryo transfer may occur in spite of normal precautions. (AM J OBSTET GYNECOL 1992;166:148-9.)

Key words: Embryo transfer, pelvic infection, embryo donation

Complications in women undergoing intrauterine catheterization for embryo transfer are exceedingly rare.[1] In most cases patients undergo ovarian aspiration before embryo transfer, which makes it difficult to determine the contribution of the prior procedure in the overall risk assessment. We report the occurrence of a severe pelvic infection resulting in a tuboovarian abscess after embryo donation to an agonadal woman. This case illustrates that infection may result from transcervical embryo transfer in spite of precautions taken in the performance of this procedure.

Case report

The patient was a 38-year-old, nulligravid, Asian woman who was previously diagnosed with premature ovarian failure. She was married and sexually active with her husband. Semen culture before oocyte donation was negative for pathogens. Fifteen years previously she underwent a right salpingo-oophorectomy after a pelvic infection. She had no history of pelvic infection, surgery, or abdominal pain since that time. A late luteal phase endometrial biopsy was performed 2 months before the planned oocyte donation while the patient was receiving exogenous sex steroid replacement as previously described.[2] The biopsy specimen was within normal limits, and the patient then underwent hormonal synchronization with the menstrual cycle of a 32-year-old woman who underwent controlled ovarian hyperstimulation and oocyte aspiration for the sole purpose of gamete donation. The donor provided 12 oocytes, resulting in the creation of five preembryos. Forty-eight hours after the aspiration the preembryos were transferred transcervically with a 3Fr tomcat catheter. This catheter has been extensively used in our program for this purpose and for the intrauterine insemination of washed sperm. It is flexible, easy to use, sterile, and known not to be embryo toxic. The transfer was performed with aseptic technique and proceeded without difficulty or incident. A tenaculum was not used on the cervix. Prophylactic antibiotics were not prescribed, nor are they routinely used by us for patients undergoing either intrauterine catheterization for insemination or for agonadal recipients needing embryo donation.

Approximately 72 hours after embryo transfer the patient experienced lower abdominal pain and fever. She was seen by her local doctor, who thought she might have a viral infection and sent her home. Her symptoms worsened and 6 days after transfer she returned to our office. Her temperature was 102° F, and she had severe lower abdominal pain and distention. Transvaginal ultrasonography noted a 6 cm × 6 cm left-sided pelvic mass, not previously noted on ultrasonography performed at the time of endometrial biopsy. The white blood cell count was 19,700/mm³ with a shift to the left. A diagnosis of tuboovarian abscess was made, and she was admitted to the hospital. Intravenous antibiotics consisting of ampicillin, gentamicin, and clindamycin were begun. After 36 hours of triple antibiotics she remained febrile to 102° F, and the abdominal distension worsened. The white blood cell count was 22,000/mm³, and it was elected to perform an operation for extirpation of the abscessed tissue. A left salpingo-oophorectomy was performed and a large abscess drained. Hormonal replacement medications were discontinued, and she menstruated. The patient did well, postoperatively, and she was afebrile 72 hours later. She was discharged on the sixth postoperative day and recovered uneventfully.

Comment

This case illustrates the potential for an atraumatic embryo transfer to result in severe pelvic infection. Although the likelihood of such an untoward outcome is exceedingly small, patients should be informed of such a complication before their consent to the procedure.

It remains uncertain whether the use of prophylactic antibiotics would prevent such an occurrence. At this time we cannot recommend routine antibiotic usage since this complication to date remains unreported.

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However, as a result of our experience, we now offer prophylaxis to patients undergoing transcervical catheterization if the patient gives us a history of pelvic infection. Similarly, in cases of traumatic catheterization of the cervix during embryo transfer or intrauterine insemination procedures, antibiotic coverage should be considered.

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Urinary excretion of prostacyclin and thromboxane metabolites in threatened preterm labor: Effect of indomethacin and nylidrin

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OBJECTIVE: We studied the role of smooth muscle–relaxing prostacyclin and its endogenous antagonist, thromboxane A_2, in preterm labor by assessing the urinary output of the breakdown products of prostacyclin (6-keto-prostaglandin $F_{1\alpha}$ and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$) and those of thromboxane A_2 (thromboxane B_2, 2,3-dinor-thromboxane B_2).
STUDY DESIGN: Thirty-three women in preterm labor between 25 and 34 weeks of gestation were studied before, during, and after treatment with indomethacin (n = 16) or nylidrin (n = 17). Urinary prostanoid levels were determined by high-performance liquid chromatography followed by radioimmunoassay, and the excretion was expressed as nanograms of prostanoids per millimole of creatinine. Statistical analyses were done by paired and unpaired Student t test, by Spearman's correlation, and by Wilcoxon signed-rank test.
RESULTS: Preterm labor was accompanied by a median 32% higher output of prostacyclin and thromboxane A_2 metabolites as compared with those in 25 controls. At 8 hours after the start of treatment indomethacin induced maximal drops in 6-keto-prostaglandin $F_{1\alpha}$ (70%), in dinor-6-keto-prostaglandin $F_{1\alpha}$ (60%), in thromboxane B_2 (85%), and in dinor-thromboxane B_2 (95%) excretion. Within 1 week after the cessation of indomethacin, output of prostacyclin metabolites had recovered to pretreatment values, whereas output of thromboxane A_2 metabolites was yet lower than the pretreatment value. Nylidrin induced no change in the output of prostacyclin and thromboxane A_2 metabolites.
CONCLUSION: Threatened preterm labor is associated with a rise in prostacyclin and thromboxane A_2 synthesis. Indomethacin inhibits more thromboxane A_2 than does prostacyclin synthesis. These findings may explain the fetal vascular changes during maternal indomethacin treatment. (AM J OBSTET GYNECOL 1992;166:150-4.)

Key words: Preterm labor, urinary prostaglandins, tocolysis

During the last decade it has become clear that antiaggregatory and vasodilatory prostacyclin (PGI_2) and its endogenous antagonist, thromboxane A_2 (TxA_2), play a significant role in the physiology of pregnancy.[1-3] Hypertensive pregnancies are characterized with PGI_2 deficiency or TxA_2 overproduction, and these changes may lead to vasoconstriction, microthromboses, and diminished blood flow in the fetoplacental unit.[1-3] Further support for the significance of the PGI_2/TxA_2 balance in pregnancy comes from a trial demonstrating that doses of aspirin so small that they inhibit only the synthesis of TxA_2, leaving PGI_2 unaffected, protect against the development of preeclampsia and other hypertensive pregnancy complications.[4]

Whereas the significance of PGI_2 and TxA_2 in hypertensive pregnancies appears clear, data are scanty on PGI_2 and TxA_2 in preterm labor. PGI_2 relaxes and TxA_2 constricts the human myometrium in in vitro study models.[5] Furthermore, it is known that labor is associated with the release of prostaglandins (PG) and prostanoids from the fetal membranes, the decidua, or the fetus itself.[6-8] Additional support for the role of prostanoids in labor comes from data indicating that PG synthesis inhibitors, such as indomethacin, arrest preterm labor.[9, 10] This treatment may, however, cause a transient constriction of the ductus arteriosus[11] and cerebral arteries,[12] and this could well be a result of PGI_2 suppression in the fetal vasculature, particularly because indomethacin is known to cross the placenta.[13] However, no direct data exist on the effect of indomethacin on PGI_2 and TxA_2 in pregnancy. Furthermore, the use of β-sympathomimetic agents, another tocolytic alternative, is accompanied by vascular

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Table I. Clinical characteristics of study population

	Preterm labor (n = 33)	*Gestational age-matched preterm controls (n = 25)*	*Term labor (n = 28)*
Age (yr)			
Median	30.1	30.0	28.0
Range	23-40	21-43	21-41
Gestational age at study (wk)			
Median	30.0	30.1	39.0
Range	25.0-34.0	25.2-34.1	37.0-42.0
Gestational age at delivery (wk)			
Median	37.2[a]	39.0[b]	39.0
Range	25.2-41.1	37.0-41.0	37.0-42.0
Birth weight (gm)			
Median	2830.0[c]	3480.0[d]	3780.0
Range	720.0-4100.0	2820.0-3980.0	2550.0-4440.0

Wilcoxon test, $p < 0.05$, *a* compared with *b* and *c* compared with *d*.

changes such as hypotension where changes in PGI_2 have been supposed to be significant.[10, 14] We therefore studied the production of PGI_2 and TxA_2 in preterm labor and the effect of indomethacin and nylidrin on these productions.

Material and methods

With the approval of the local ethical committee, 86 pregnant women were studied (Table I). Of these, 33 otherwise healthy women were admitted to the hospital because of an established threat of preterm labor between 25 and 34 gestational weeks of singleton pregnancy. They all had regular painful uterine contractions at <10-minute intervals, and the cervix was softened and opened (2 to ≤4 cm) (median Bishop score, 6; range, 2 to 8). The fetal membranes were intact.

As the control group for these women with threatened preterm labor, we studied 25 healthy pregnant women experiencing no uterine contractions who were matched for the gestational age (±3 days). To learn whether preterm and term uterine activity was differently associated with PGI_2 and TxA_2, we studied another control group of 28 women who had been in term labor for 3 to 12 hours and whose cervices were open 2 to 10 cm (median, 3 cm) (Table I).

The 33 women were threatened preterm labor were treated in randomized order (a sealed envelope) with indomethacin (16 women) or nylidrin (17 women). Three days of an indomethacin course consisted of an initial 100 mg rectal suppository followed by two 50 mg oral capsules at 8-hour intervals for the first day; on days 2 and 3 the dose was 50 mg orally, three times a day. Nylidrin was infused intravenously with an initial dose of 50 μg/min; this was increased within 30 minutes to the maximum of 150 μg/min and continued for 3 days. The double-dummy principle was followed so that all patients received both the intravenous infusion (containing placebo or nylidrin) or rectal suppositories and oral capsules (containing indomethacin or placebo).

Maternal blood pressure and pulse rate were recorded hourly up to 8 hours and then 12, 24, 48, and 72 hours after initiation of treatment. Fetal heart rate was monitored by continuous cardiotocography. Labor was halted for >24 hours in all 33 patients and postponed for 2 to 7 days in nine patients (four indomethacin, five nylidrin), for 1 to 2 weeks in four patients (1 indomethacin, 3 nylidrin), and for >2 weeks in 20 patients (11 indomethacin, 9 nylidrin). More detailed clinical data will appear in a subsequent article.[15]

Spot urinary samples (50 ml) were collected before the start of the indomethacin or nylidrin from women with preterm labor. Similar urine samples were collected 8 hours (from 12 women), 24 hours (from all women), 72 hours, 1 week, and 2 weeks (from 14 women each) after the onset of tocolysis. The distribution of the samples collected was equal between the indomethacin and nylidrin groups. Single urine samples were collected from the control groups.

The samples were stored frozen (at −21° C) until assayed for 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ (2,3-dinor-6-keto-$PGF_{1\alpha}$), thromboxane B_2 (TxB_2), and 2,3-dinor-TxB_2 by means of high-performance liquid chromatography followed by radioimmunoassays. The details of all methods except for 2,3-dinor-TxB_2 are given elsewhere[16]; as regards 2,3-dinor-TxB_2, it eluted from high-performance liquid chromatography concomitantly with 6-keto-$PGF_{1\alpha}$ and was assayed with tritium-labeled TxB_2 as a tracer and TxB_2 antibody that has approximately 40% cross reactivity with 2,3-dinor-TxB_2. Creatinine was determined by a routine method, and the prostanoid output was expressed against creatinine excretion.

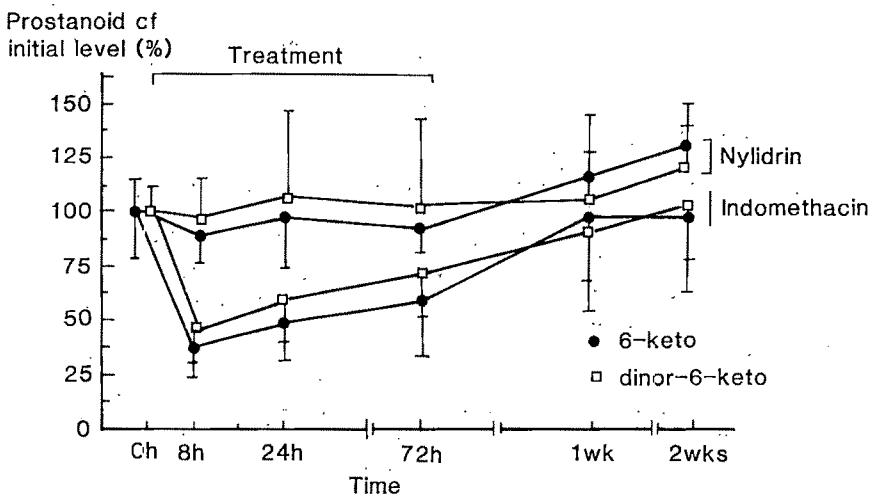


Fig. 1. Mean (± SE) urinary excretion of PGI_2 metabolites (6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$) as percentage of initial level in patients with indomethacin and nylidrin treatment. Drops were significant ($p < 0.05$) at 8, 24, and 72 hours during indomethacin treatment but insignificant during nylidrin treatment (Student t test). For numbers of observations see text.

Table II. Urinary excretion of PGI_2 (6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$) and thromboxane (TxB_2, 2,3-dinor-TxB_2) metabolites and their ratios in women in preterm labor as compared with gestational age–matched control women and women in term labor

	Preterm labor (n = 33)		*Gestational age–matched preterm controls (n = 25)*		*Term labor (n = 28)*	
	Median	*Range*	*Median*	*Range*	*Median*	*Range*
Prostanoid (ng/mmol creatinine)						
6-Keto-$PGF_{1\alpha}$	35.0[a]	13.5-136.0	32.6[b]	6.0-80.9	59.4[c]	8.5-207.4
2,3-Dinor-6-keto-$PGF_{1\alpha}$	44.8[d]	15.9-120.5	38.5[e]	8.1-107.1	40.0	15.6-122.0
TxB_2	17.5[f]	2.6-105.9	11.1[g]	0.7-30.6	18.7	4.2-272.9
2,3-Dinor- TxB_2	77.4[h]	12.1-383.5	54.2[i]	1.7-143.7	73.2	24.2-333.3
$\frac{\text{6-Keto-PGF}_{1\alpha}}{\text{TxB}_2}$	2.3[j]	0.6-8.5	3.4[k]	0.4-14.7	2.7	0.4-12.1
$\frac{\text{2,3-Dinor-6-keto-PGF}_{1\alpha}}{\text{2,3-Dinor-TxB}_2}$	0.6[l]	0.1-2.4	0.8[m]	0.1-4.8	0.6	0.1-2.3
$\frac{\text{6-Keto-PGF}_{1\alpha} + \text{2,3-dinor-6-keto-PGF}_{1\alpha}}{\text{TxB}_2 + \text{2,3-dinor-TxB}_2}$	0.9[n]	0.1-2.9	1.1[o]	0.2-7.6	1.1	0.2-3.5

Wilcoxon test, $p < 0.05$, *a* compared with *b*, *a* with *c*, *d* with *e*, *f* with *g*, and *h* with *i*. Student t test, $p < 0.05$, *j* compared with *k*, *l*, with *m*, and *n* with *o*.

Statistical analyses were done by paired and unpaired Student t test for parametric data, by Spearman's correlation, and by Wilcoxon signed rank test for nonparametric data. In addition to the individual prostanoid output, PGI_2 metabolites (6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$) and TxA_2 metabolites (TxB_2 and 2,3-dinor-TxB_2) also were summarized to reflect the total production of the parent compound. To better demonstrate any possible treatment-induced changes in prostanoid excretions, they were also expressed as percentages of the initial output.

Results

Preterm labor was accompanied by increased outputs of all prostanoids measured (Table II). The ratio of PGI_2 to TxA_2 metabolites was approximately 36% lower for preterm labor women than for preterm control women (Table II). No relationship was evident between individual prostanoid excretion and days gained by tocolysis, nor there was any difference in prostanoid output between the women ($n = 15$) who subsequently gave birth before the thirty-seventh gestational week and those who proceeded to term ($n = 18$).

There also was no difference in the pretreatment output of prostanoid metabolites between the indomethacin and nylidrin groups (detailed analysis not shown). Indomethacin treatment was accompanied by maximal drops in 6-keto-$PGF_{1\alpha}$ (70% ± 9.1%, SE) and 2,3-dinor-6-keto-$PGF_{1\alpha}$ (60% ± 9.4%, SE) and by maximal drops in TxB_2 (85% ± 8.6%, SE) and 2,3-dinor-

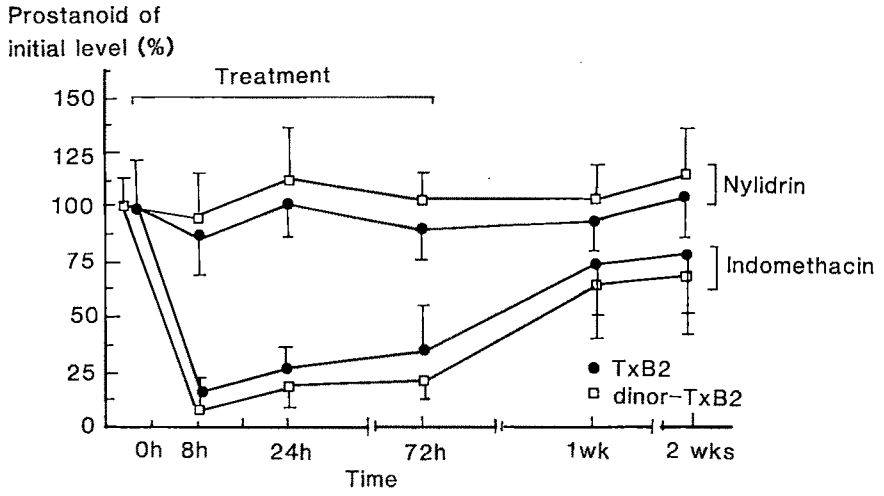


Fig. 2. Mean (±SE) urinary excretion of TxA_2 metabolites (TxB_2 and 2,3-dinor-TxB_2) as percentage of initial level in patients with indomethacin and nylidrin treatment. Drops were significant ($p < 0.05$) at 8, 24, and 72 hours during indomethacin treatment but insignificant during nylidrin treatment (Student t test). For numbers of observations see text.

TxB_2 excretion (95% ± 7.9%, SE) at 8 hours after the start of the tocolysis, whereas nylidrin induced no significant changes (Figs. 1 and 2). Indomethacin treatment decreased TxA_2 (TxB_2 and 2,3-dinor-TxB_2) approximately 35% more ($p < 0.05$) than it did PGI_2 (6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6-keto-$PGF_{1\alpha}$) excretion, resulting in an increased ratio of PGI_2 to TxA_2 metabolites from 1.2 ± 0.2 to the maximum of 3.4 ± 0.7 (SE) ($p < 0.05$) within 8 to 72 hours after the start of indomethacin (Figs. 1 and 2). Within 1 week after cessation of indomethacin, the output of PGI_2 metabolites had recovered to pretreatment levels (Fig. 1), whereas output of TxA_2 metabolites remained lower than the pretreatment value (Fig. 2).

During term labor the excretion of these prostanoids increased in relation to cervical dilatation: 6-keto-$PGF_{1\alpha}$ ($r = 0.6$, $p = 0.001$), 2,3-dinor-6-keto-$PGF_{1\alpha}$ ($r = 0.4$, $p = 0.044$), TxB_2 ($r = 0.6$, $p = 0.002$), and 2,3-dinor-TxB_2 ($r = 0.5$, $p = 0.008$). When the individual prostanoid output figures during term labor were considered in isolation from cervical dilatation, term labor was accompanied by a higher ($p < 0.05$) output of 6-keto-$PGF_{1\alpha}$ (median, 59.4; range, 8.5 to 207.4 ng/mmol creatinine) than was preterm labor (median, 35.0; range, 13.5 to 136.0), whereas all the other prostanoid output figures and the ratio of PGI_2 to TxA_2 metabolites did not show any difference between women in term and preterm labor.

During the first 8 hours of treatment nylidrin induced a significant drop in diastolic blood pressure from a median of 60 (range, 50 to 80) to a median of 50 (range, 30 to 70) mm Hg; this change disappeared after 8 hours. The magnitude of the fall in diastolic blood pressure was of no relation to PGI_2 or TxA_2 output. Indomethacin induced no changes in blood pressure.

Comment

At present the in vivo production of PGI_2 and TxA_2 is measured by determining the urinary metabolites of the parent compounds, although it is not totally clear to what extent they reflect systemic and to what extent renal prostanoid synthesis.[17, 18] The best index of systemic PGI_2 production is considered to be urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$, and 2,3-dinor-TxB_2 is the best index of TxA_2 production by circulating platelets,[19] whereas the majority of 6-keto-$PGF_{1\alpha}$ and TxB_2 may arise from the kidneys.[20] In our study we assessed all four of these prostanoids.

Abundant evidence suggests a role for prostaglandins in the initiation of term human labor.[6-8] Thus activation of arachidonic acid metabolism also could induce preterm labor, although so far there has been little in vivo evidence to support this claim. In the only earlier work Noort[21] found increased amounts of urinary TxB_2 in women in imminent preterm labor. This was confirmed in our study, although we could not demonstrate any value for TxB_2 assay in predicting the outcome of tocolysis in individual patients. As a new finding, the excretion of 2,3-dinor-TxB_2, 6-keto-$PGF_{1\alpha}$, and 2,3-dinor-6-keto-$PGF_{1\alpha}$ also was elevated in threatened preterm labor. Premature uterine activity thus seems to be associated with general stimulation in prostanoid synthesis. However, our current data do not reveal whether this stimulation is a primary change, perhaps connected to the onset of contractions, or a secondary change resulting from the compliance of the uterus and vascular system to preterm contractions. In theory the dominance of the production of contraction/promoting TxA_2,[5] as evident in our study, could be involved in the onset or maintenance of contractions.

Maternal indomethacin treatment caused a transient constriction of the ductus arteriosus in seven of 14

fetuses[11] and of cerebral arteries in six of 13 fetuses.[12] In view of the significant role of PGI_2 in ductal patency,[1-3] PGI_2 suppression is a probable explanation, although no in vivo data on the effect of indomethacin on PGI_2 during pregnancy have been available. We present now the first in vivo evidence that maternal indomethacin suppresses the synthesis of PGI_2, although we do not know the exact site where indomethacin takes this action. It may inhibit PGI_2 on both the maternal and the fetal side,[13] and the suppression in fetal PGI_2 synthesis may be one explanation for the fetal vessels' constriction.[11, 12] PGI_2 synthesis did recover within 1 week after indomethacin treatment, which fits in with the findings that ductal constriction vanishes within the same time after cessation of indomethacin.[11, 22]

β-Sympathomimetic-induced vasodilatation often has been supposed to be due to the excess of PGI_2,[10] and this was supported by small rises in maternal plasma 6-keto-$PGF_{1\alpha}$ after ritodrine infusion.[14] As assessed by its urinary metabolites, nylidrin did not increase PGI_2 production, although a significant fall in diastolic blood pressure ensued. Thus nylidrin and probably also the other β-sympathomimetics are unlikely to affect PGI_2 synthesis in vivo. Also in vitro models β-sympathomimetics had no effect on PGI_2 synthesis.[23] That nylidrin does not affect PGI_2 synthesis in vivo is a finding in harmony with unchanged ductal flow during maternal nylidrin infusion.[22]

We conclude that threatened preterm labor is associated with increased PGI_2 and TxA_2 synthesis and with TxA_2 dominance. Indomethacin suppresses the production of PGI_2 and, even more, that of TxA_2, which may be one reason for the tocolytic efficacy of indomethacin. However, nylidrin has no effect on these prostanoids. Inhibition of fetal PGI_2 synthesis during maternal indomethacin administration may be one reason for the ductal constriction during such a treatment.

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Substance P in peritoneal fluid

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Substance P is a neuropeptide that has been identified in the ovary, fallopian tube, uterus, and vagina and in the hypothalamic-pituitary axis in both an animal model and human ovaries. We sought to determine if substance P is present in peritoneal fluid and, if so, whether it correlated with the cause of infertility. Its presence was determined by radioimmunoassay in the peritoneal fluid of 66 patients undergoing diagnostic laparoscopy for clinical indications related to infertility. Total volume of peritoneal fluid and cycle day were recorded; patients were evaluated in groups according to diagnosis: endometriosis (n = 24), pelvic adhesions (n = 18), and normal controls (n = 24). The level of substance P (mean ± SEM) was 122 ± 19 pg/ml for endometriosis and 130 ± 19 pg/ml for pelvic adhesions. These values were not significantly different from the normal controls (130 ± 25 pg/ml). There was no significant difference in levels between follicular and luteal phase of the menstrual cycle. We conclude that substance P is present normally in peritoneal fluid and that its levels are not affected by pelvic endometriosis or adhesions. (Am J Obstet Gynecol 1992;166:155-9.)

Key words: Substance P, peptides, peritoneal cavity, endometriosis

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6/1/32392

Substance P is a brain-gut peptide consisting of 11 amino acids and belonging to the family of tachykinins. In the brain it behaves as a neuropeptide and may have a physiologic role as an inhibitor of gonadotropin-releasing hormone.[1] In the gastrointestinal and urogenital tracts substance P has a stimulatory effect on smooth muscles. The neuropeptide also acts on the

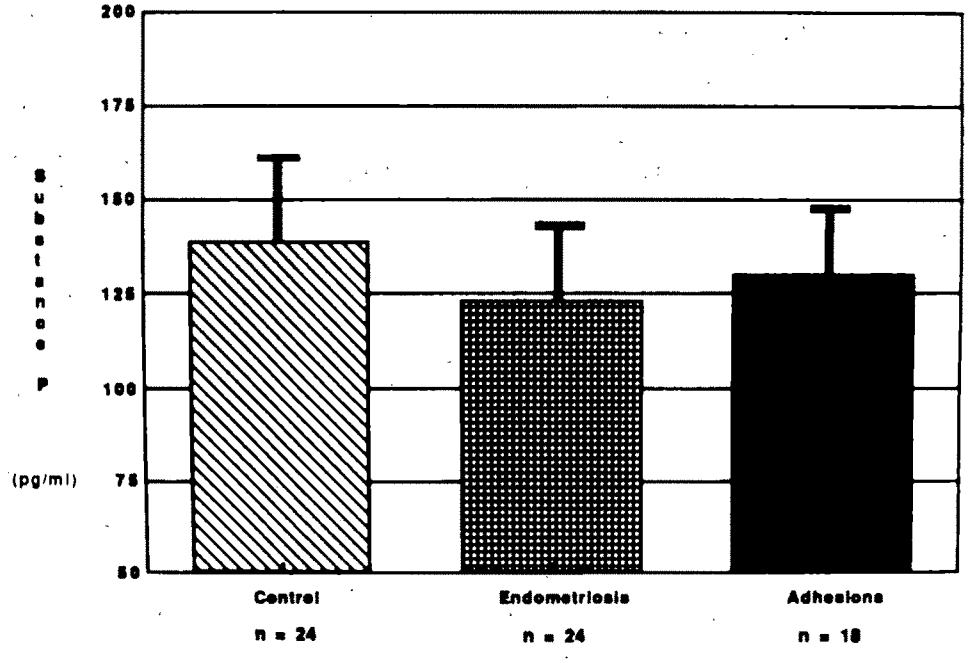


Fig. 1. Substance P in peritoneal fluid for patients with endometriosis and adhesions and control population. (Substance P levels are expressed in picograms per milliliter.)

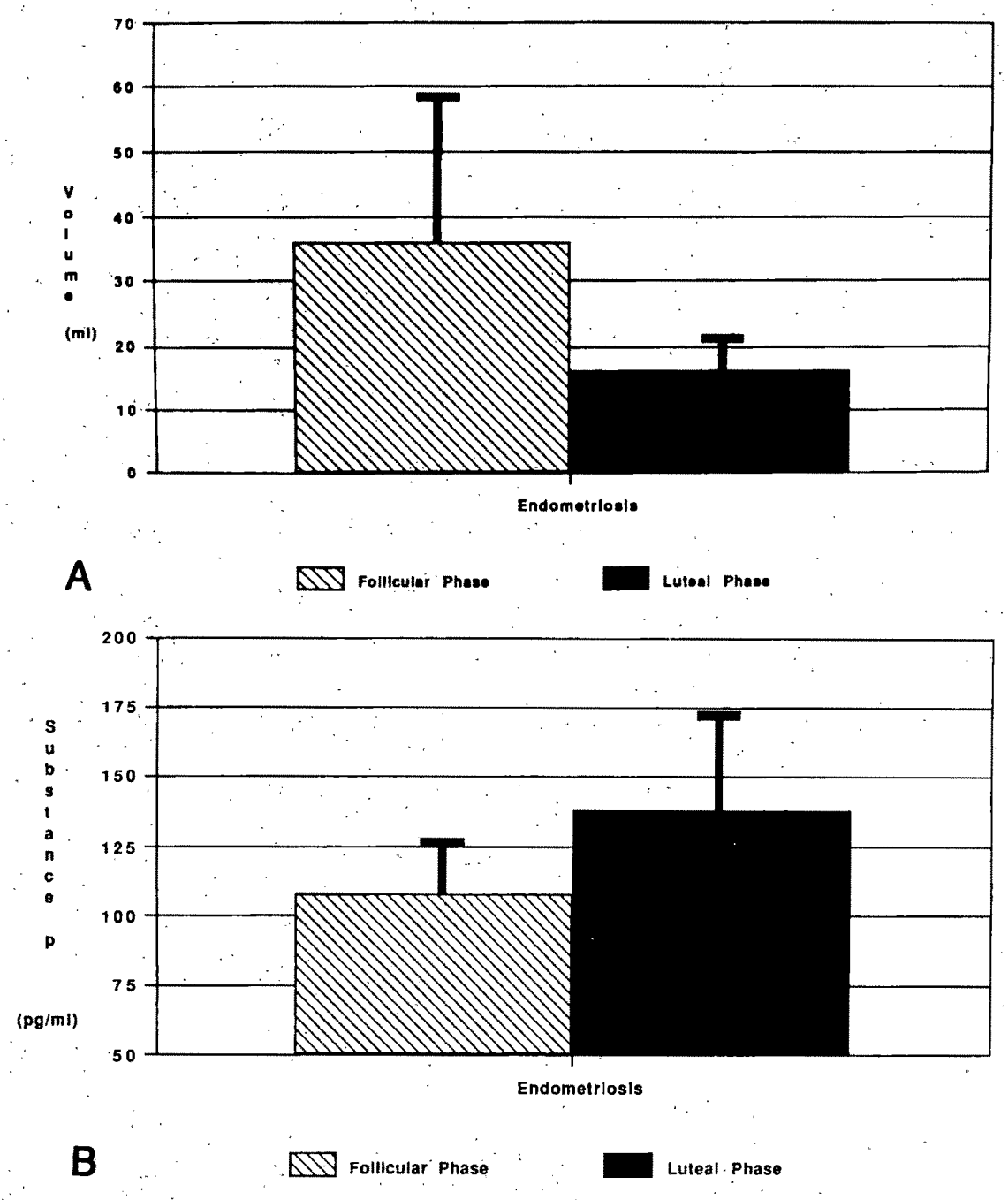


Fig. 2. A, Comparison of peritoneal fluid volumes in follicular versus luteal phases of menstrual cycle in patients with endometriosis. **B,** Substance P levels in endometriosis population, follicular versus luteal phase.

smooth muscles of blood vessels, resulting in vasodilatation.[2]

Substance P has been isolated from the ovaries, fallopian tubes, uterus, and hypothalamic-pituitary axis in animal models.[2] It also has been isolated from human ovaries and is found in high concentrations in primary ovarian carcinoid.[3]

The fallopian tube is extremely sensitive to tachykinins. Exposure of fallopian tubes to tachykinins causes an increase in tubal motility. It has been suggested that the increased tubal motility that occurs during ovulation could be mediated by the local release of tachykinins.[4]

The objective of this study was to determine if substance P normally is present in peritoneal fluid and, if so, whether its level is affected by the presence of pelvic pathologic conditions.

Material and methods

Sixty-six patients were evaluated. Each individual underwent diagnostic laparoscopy for evaluation of infertility, sterilization, or chronic pelvic pain and con-

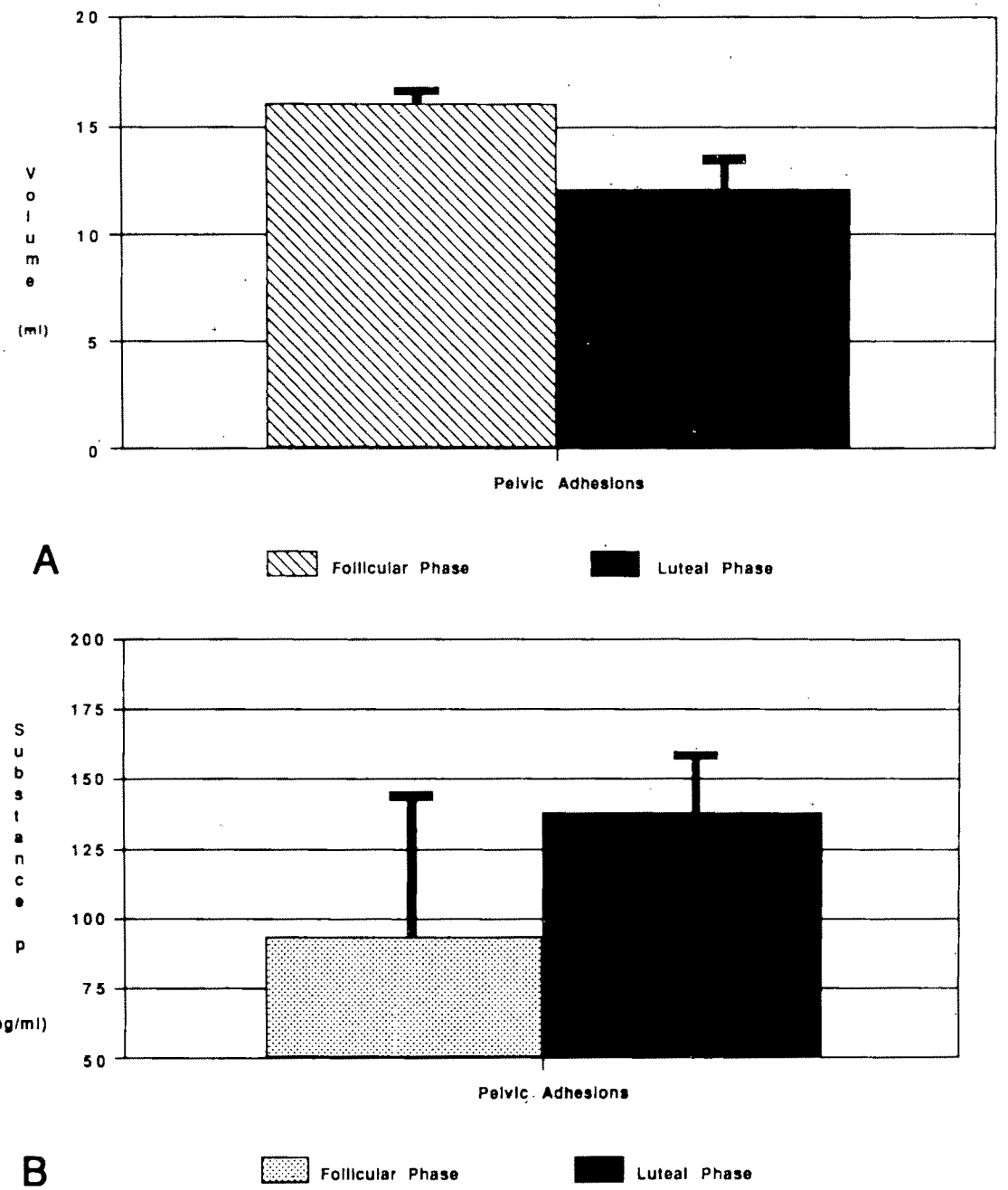
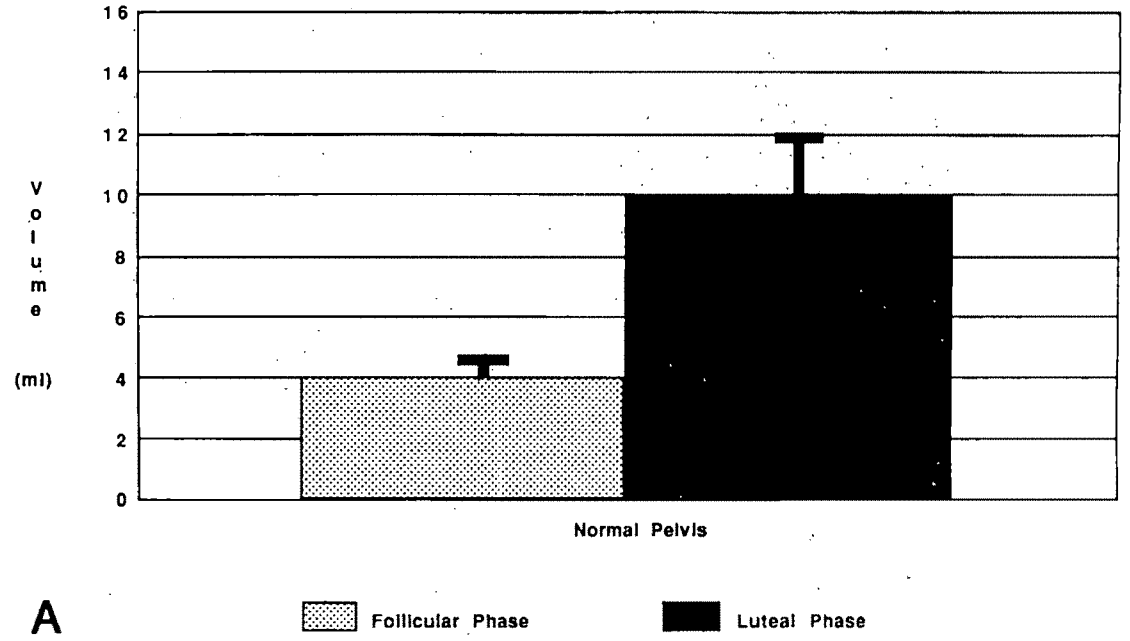


Fig. 3. A, Comparison of peritoneal fluid volumes in follicular versus luteal phase of menstrual cycle in patients with pelvic adhesions. **B,** Substance P levels in pelvic adhesion population, follicular versus luteal phase.

sented to removal of peritoneal fluid for analysis of substance P. Prior institutional review board approval was obtained. Each patient's menstrual cycle day, total peritoneal fluid volume, and intraoperative findings were recorded. If endometriosis (n = 24) was present, it was classified according to the American Fertility Society classification revised in 1988. Adhesions were present in 10 patients, and all were filmy and avascular. Patients with normal pelvic findings (n = 24) served as controls. Phase of the menstrual cycle and total volume of peritoneal fluid were determined.

Radioimmunoassay for substance P. After measuring the total volume of fluid obtained, an aliquot of fresh peritoneal fluid was placed in an ultracold (−70° C) freezer until the assay was performed. The samples were thawed in an ice bath (0° C). An acetone extraction of substance P was obtained from peritoneal fluid.

Radioimmunoassay of substance P was carried out with kits and protocols supplied by the Immuno Nuclear Corporation, Stillwater, Minn. In essence, rabbit anti–substance P serum and iodine 125–substance P was used with both substance P standards and perito-



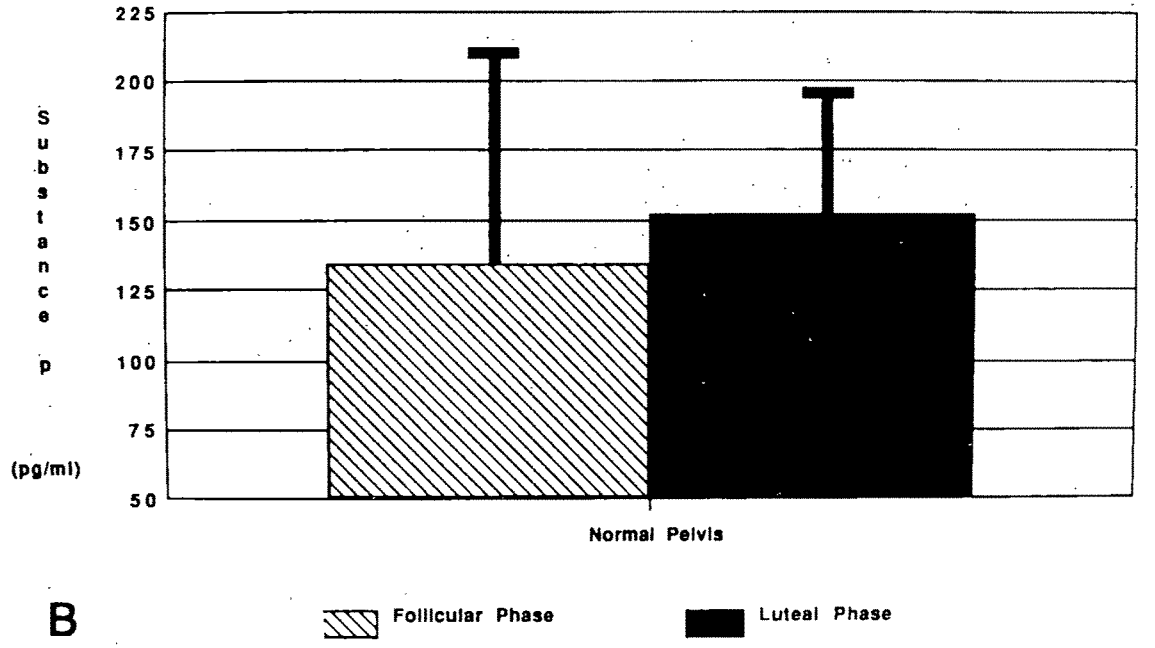


Fig. 4. A, Comparison of peritoneal fluid volumes in follicular versus luteal phase of menstrual cycle in control patient population. **B,** Substance P levels in control population, follicular versus luteal phase.

neal fluid samples (duplicates of each standard and sample).

After incubation, rabbit γ-globulin was added to all tubes, followed by saturated ammonium sulfate, followed by a second incubation. The supernatant was aspirated, and the precipitate of each tube was counted in a γ-scintillation counter. To calculate the percent bound per total count, the following formula was used:

$$\% \text{ Bound/total count} = \frac{\text{counts/min of standard or sample}}{\text{counts/min of total count tube}}$$

A standard curve was prepared by plotting the percent bound on the ordinate and the concentration of the standards in picograms per milliliter on the abscissa. The substance P value was read off the standard curve.

The assay was tested for cross-reactivity with related peptides—methionine enkephalin, leucine enkephalin, β-endorphin, eledoisin, and physalaemin. The interassay coefficient of variation equaled 17.3%, and the intraassay coefficient of variation equaled 7.8%. The assay resulted in a 0.008% cross reactivity with the peptides. Analysis of variance tests were used for statistical analysis.

Results

Patients with endometriosis ($n = 24$) were classified as stage 1 ($n = 2$), stage 2 ($n = 16$), stage 3 ($n = 2$), or stage 4 ($n = 4$). There was no significant difference in substance P levels among stages 1 to 4 of endometriosis. The levels of substance P (expressed in picograms per milliliter, mean ± SEM) in peritoneal fluid for patients with endometriosis (122 ± 19 pg/ml), pelvic adhesions (130 ± 19 pg/ml), and controls (139 ± 25 pg/ml) were not significantly different (Fig. 1).

Levels of substance P in peritoneal fluid were compared in all groups by menstrual phase. There was no significant difference noted between substance P level in the proliferative phase (104 ± 20 pg/ml) and luteal phase (137 ± 17 pg/ml) among study patients nor was there a statistical difference in total volume of fluid between proliferative and luteal phase with the three patient groups: endometriosis 12.6 ± 3.0 ml adhesions 16.5 ± 0.9 ml, and control 5.0 ± 0.7 ml for the proliferative phase and endometriosis 15.8 ± 4.6 ml, adhesions 13.3 ± 2.2 ml, and control 9.8 ± 2.4 ml for the luteal phase (Figs. 2 to 4).

Comment

It has been theorized that the presence of substance P in peritoneal fluid may play a role in regulating tubal motility during ovulation, and alterations in the levels of substance P may alter tubal motility in pathologic conditions.[4] Alterations of substance P in peritoneal fluid associated with pelvic adhesions and endometriosis were investigated.

The levels of substance P in the peritoneum were approximately tenfold higher than in serum.[2] There was no statistical difference in substance P levels in peritoneal fluid between patients with a laparoscopically normal pelvis and patients with either pelvic adhesions or endometriosis. Nor was there a difference in the level of substance P in peritoneal fluid in the follicular phase of the menstrual cycle when compared with the luteal phase in any of the three groups.

In summary, this is a preliminary report noting the similar levels of substance P in peritoneal fluid in women with endometriosis and adhesions compared with controls. The physiologic role of such high levels of substance P in peritoneal fluid on the reproductive tract remains to be determined.

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Partial hydatidiform moles have impaired differentiated function (human chorionic gonadotropin and human placental lactogen secretion) in response to epidermal growth factor and 8-bromo-cyclic adenosine monophosphate

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OBJECTIVE: The null hypothesis is that partial hydatidiform moles have normal differentiated function (human chorionic gonadotropin and human placental lactogen secretion) in response to epidermal growth factor and 8-bromo-cyclic adenosine monophosphate.
STUDY DESIGN: Two complete moles, 10 partial hydatidiform moles, and 19 normal first-trimester placentas in monolayer culture were exposed to 10 ng/ml epidermal growth factor, 1 mmol/L 8-bromo-cyclic adenosine monophosphate plus 1 mmol/L theophylline, or control. Human chorionic gonadotropin and human placental lactogen secretion was measured. Frequency of response to stimuli was compared by χ^2 analysis, and hormone secretion was compared by analysis of variance.
RESULTS: Partial moles demonstrated reduced frequencies of response of human chorionic gonadotropin and human placental lactogen to epidermal growth factor (partial moles 2/8 and 2/8, respectively; normal placentas 16/19 and 7/18, respectively; $p < 0.025$) and of human chorionic gonadotropin to 8-bromo-cyclic adenosine monophosphate (partial moles 3/5, normal placentas 13/16; $p < 0.005$).
CONCLUSION: Partial hydatidiform moles demonstrate impaired human chorionic gonadotropin and human placental lactogen secretory responsiveness to epidermal growth factor and cyclic nucleotides in comparison with normal first-trimester trophoblast. (Am J Obstet Gynecol 1992;166:160-6.)

Key words: Hydatidiform mole, human chorionic gonadotropin, human placental lactogen, epidermal growth factor, growth factors

Partial and complete hydatidiform moles, which are characterized by inappropriate hyperplasia of the villous tissue, demonstrate a variety of morphologic, antigenic, cytogenetic, and hormonal abnormalities.[1-13] Although β-human chorionic gonadotropin (hCG) has been most useful as a disease marker, no information is available regarding the regulation of hCG or other hormones produced by molar tissue. Therefore we wished to determine if there was abnormal regulation of hCG and human placental lactogen (hPL) secretion in response to epidermal growth factor (EGF) and 8-bromo-cyclic adenosine monophosphate (8-bromo-cAMP), two known stimulators of hormone secretion in normal trophoblast.

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6/1/30965

Material and methods

The study protocol was approved by the institutional ethics review board. Normal first-trimester placentas and hydatidiform mole tissue from spontaneous or therapeutic abortions were obtained and transported in sterile saline solution to the laboratory. Membranes were removed and the entire piece of placenta without dissection was then subjected to eight sequential 10-minute treatments with 0.25% trypsin/10 U/ml deoxyribonuclease I as previously described.[14] Cells were collected after the third to eighth treatment, pooled, centrifuged at 300*g*, and resuspended in Dulbecco's modified Eagle medium containing 10% fetal calf serum and 50 U/ml penicillin, 50 μg/ml streptomycin, and 0.2 mmol/L glutamine. Cells were plated in 60 mm Petri dishes and allowed to attach for 2 hours, after which the medium was changed to Dulbecco's modified Eagle medium without serum for the remainder of the experiment (7 to 9 days). Medium was changed daily and frozen at −20° C for subsequent hCG and hPL assay. After each medium change, plates received either 10 ng/ml EGF or bovine serum albumin (final concen-

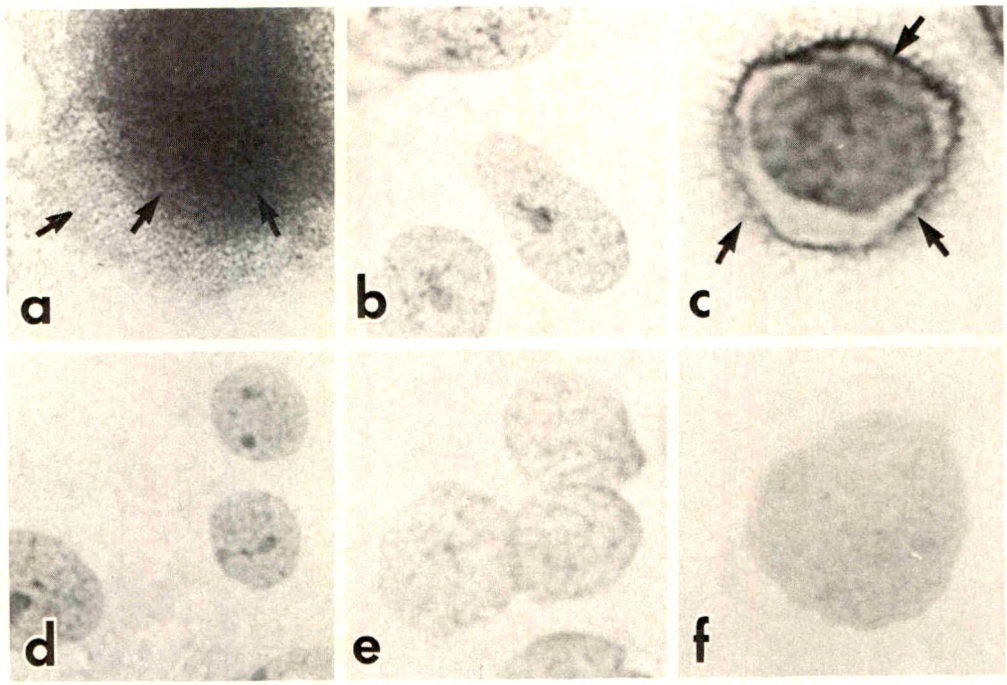


Fig. 1. Immunoperoxidase-ABC staining of partial hydatidiform mole. All cells used were cytospun samples of isolated cell preparations. **a,** $(Fab')_2$ fragment of oncomodulin showing positive staining *(arrows)* for cytotrophoblast; **b,** absent GZ100 staining, indicating no syncytial contamination of isolated cell preparations; **c,** positive cytokeratin staining *(arrows)*, indicating trophoblasts, **d,** absence of HLe-1 staining, indicating no macrophage contamination; **e,** absence of vimentin staining, indicating no fibroblasts; **f,** negative control. (Original magnification ×1250.)

tration 0.001%) for the duration of the culture period. To determine hCG responsiveness, 1 mmol/L 8-bromo-cAMP/1 mmol/L theophylline was added to separate plates on culture days 2 to 4. On days 6 to 9 cells were removed from some plates with 0.25% trypsin/0.02 mol/L ethylenediaminetetraacetic acid and counted on a hemocytometer.

Cytospun samples of freshly dispersed normal first-trimester and molar cells were stained by immunoperoxidase-ABC staining procedures as previously described[14] with monoclonal antisera directed against vimentin, cytokeratin, macrophages (HLe-1), and the $F(ab')_2$ fragments of oncomodulin, which were generated with a kit (ImmunoPur Fab Preparation Kit) from Pierce, Rockford, Ill. A syncytiotrophoblast-specific antiserum (GZ100) was also tested.[15] Incubation time was 72 hours at 4° C for oncomodulin and 4 hours at room temperature for all other antisera.

The gestational age of normal and molar placentas was calculated from date of last menstrual period.

Placentas were classified as responders or nonresponders to EGF and 8-bromo-cAMP/theophylline stimulation. A responder to EGF stimulation was defined as a placenta in which the integrated hormone secretion for 7 days of culture was significantly greater than control by paired *t* test. A responder to 8-bromo-cAMP/theophylline secretion was similarly defined, but only the 3 days of 8-bromo-cAMP/theophylline exposure were used in calculations. Comparison of the frequencies of stimulation between moles and first-trimester placentas was performed with Fisher's exact test or χ^2 analysis. Other comparisons were done with analysis of variance.

Trypsin was obtained from ICN Biomedicals Canada, Ltd., Montreal; deoxyribonuclease I, 8-bromo-cAMP, theophylline, Dulbecco's modified Eagle medium, ethylenediaminetetraacetic acid, penicillin-streptomycin, glutamine, and mouse EGF were obtained from Sigma Chemical Co., St. Louis; Petri dishes were from Corning Glass Co., Corning, N.Y.; and bovine serum albumin was from Miles Laboratories, Inc., Elkhart, Ind. Antisera were from Dimension Laboratories, Mississauga, Ontario (vimentin [Monosan]), and Becton-Dickinson Immunocytometry Systems, Mountain View, Calif. (cytokeratin, HLe-1). Oncomodulin polyclonal antiserum was kindly provided by Dr. J.P.

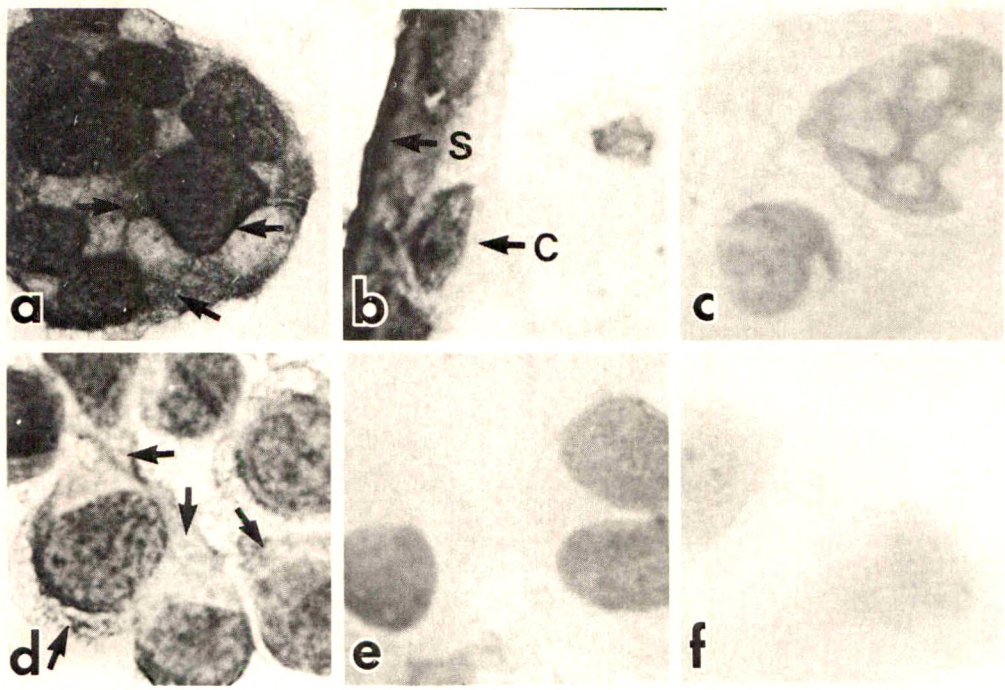


Fig. 2. Immunoperoxidase-ABC staining of normal first-trimester placenta. All cells were cytospun samples of isolated cell preparations except for **b** (frozen section of intact placenta). **a,** $F(ab')_2$ fragment of oncomodulin showing positive staining *(arrows)* for cytotrophoblast; **b,** positive GZ100 staining only in syncytiotrophoblast of intact placenta *(arrows; S,* syncytium; *C,* cytotrophoblast); **c,** absent GZ100 staining, indicating no syncytial contamination of isolated cell preparations; **d,** positive cytokeratin staining *(arrows),* indicating trophoblasts; **e,** absence of HLe-1 staining, indicating no macrophage contamination; **f,** negative control. (Original magnification ×1250.)

Table I. hCG and hPL secretion in response to 8-bromo-cAMP/theophylline and EGF stimulation

	1 mmol/L 8-Bromo-cAMP/1 mmol/L theophylline		*10 ng/ml EGF*			
			hCG		*hPL*	
	No.	*hCG fold increase**	*No.*	*Fold increase**	*No.*	*Fold increase**
Partial moles						
Responders	5	4.24 ± 1.04†	2	1.43 ± 0.22	2	2.04 ± 0.56†
Nonresponders	3	1.24 ± 0.54	8	1.23 ± 0.10	8	0.90 ± 0.07
Complete moles						
Responders	1	2.85 ± 0.58	2	1.55 ± 0.47	0	—
Nonresponders	1	1.07 ± 0.09	0	—	2	0.84 ± 0.13
Normal placenta						
Responders	13	4.21 ± 0.48‡	16	2.23 ± 0.26‡	7	1.47 ± 0.23†
Nonresponders	3	1.30 ± 0.57	3	1.06 ± 0.09	11	0.93 ± 0.08

*Mean ± SEM fold (ratio of EGF/control or 8-bromo-cAMP/theophylline/control) increase over 3 (8-bromo-cAMP/theophylline) or 7 (EGF) days of culture. Responders compared with nonresponders of same tissue type by analysis of variance.

†$p < 0.05$.

‡$p < 0.001$.

MacManus, National Research Council of Canada, Ottawa. Dr. G. Desoye, University of Graz, kindly provided the GZ100 monoclonal antiserum.

Results

Two complete and 10 partial moles were identified by standard morphologic criteria.[1] The identity and purity of the dispersed partial and complete mole and normal first-trimester cells were determined with multiple markers, as previously described[14] and are shown in Figs. 1 and 2. In the current studies two additional markers (oncomodulin and GZ100) were also used. Partial and complete moles showed positive staining for cytokeratin and oncomodulin and were negative for HLe-1 (macrophages) and vimentin (fibroblasts). An occasional vimentin-positive cell (<0.1%) was noted in

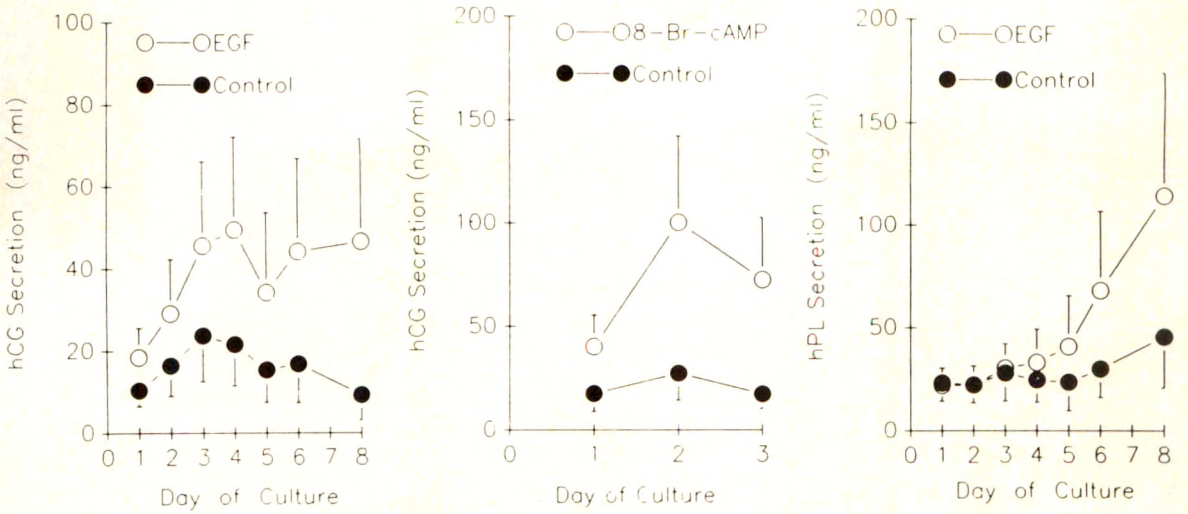


Fig. 3. Stimulation of hCG *(left panel)* and hPL *(right panel)* secretion by 10 ng/ml EGF and of hCG secretion *(middle panel)* by 1 mmol/L 8-bromo-cAMP/1 mmol/L theophylline of normal first-trimester trophoblast compared with controls. Mean ± SEM of responders appears in Table I. Responders were defined as having integrated hormone secretion over 7 culture days (EGF) or 3 culture days (8-bromo-cAMP/theophylline) that was significantly greater than control (see Material and methods). Each individual experiment (placenta) had significantly greater responses to stimuli than its own control. *Day of culture,* Culture day after EGF or 8-bromo-cAMP/theophylline was received.

the partial moles, but the one complete mole tested showed a higher percentage (10% to 15%) of vimentin-positive cells. No GZ100-positive cells were seen. GZ100 antiserum recognizes only first-trimester syncytiotrophoblast (Fig. 1, *b,* and Fig. 2, *b* and *c*).[15] Oncomodulin is a calcium-binding protein that is specific for cytotrophoblast.[16] The combination of these results indicates that the partial mole preparations were nearly pure cytotrophoblast. The complete mole was less purified. The results for normal first-trimester cytotrophoblast staining showed similar results (Fig. 2).[17] Vimentin staining was negative (data not shown), as in Fig. 1, *e,* and as reported.[17] GZ100 and oncomodulin staining of normal first-trimester trophoblast and partial and complete moles, not previously reported, permits positive identification of cytotrophoblast and exclusion of syncytial contamination. The pattern of expression of the antigens tested was the same in partial and complete moles and normal placentas.

Normal first-trimester placentas and molar tissue were collected and processed under identical conditions. Cell viability of isolated cell preparations was >95% in all preparations of both normal and molar tissue.

Table I shows the fraction of cultures that were responders or nonresponders to EGF and 8-bromo-cAMP/theophylline stimulation, using the classification of responders and nonresponders as defined in the Material and methods. By these criteria, EGF induced hCG and hPL secretion in two of eight partial moles and in 16 of 19 and seven of 18 normal first-trimester placentas, respectively. 8-Bromo-cAMP/theophylline induced hCG secretion in five of eight partial moles and 13 of 16 normal first-trimester placentas, respectively. Of the complete moles, two of two and none of two showed hCG and hPL responses to EGF, respectively, and one of two had an hCG response to 8-bromo-cAMP/theophylline. Too few complete moles were available for separate statistical comparisons with partial moles or normal placenta. Partial moles demonstrated reduced frequencies of response of hCG and hPL to EGF ($p < 0.025$, Fisher's exact test), and of hCG to 8-bromo-cAMP/theophylline ($p < 0.005$, χ^2 analysis) in comparison with normal first-trimester placenta. The mean fold or fractional increases in hormone response to EGF and 8-bromo-cAMP/theophylline (defined as EGF/control ratio for 7 culture days and 8-bromo-cAMP/theophylline/control ratio for 3 culture days) are also shown in Table I. By analysis of variance, most responders had significantly greater responses to stimuli than did nonresponders (Table I). Normal placenta and partial mole responders to EGF or 8-bromo-cAMP/theophylline did not show significant differences in the magnitude of increases of hCG or hPL (Table I). Figs. 3 and 4 show the increases in hCG and hPL secretion after EGF and 8-bromo-cAMP/theophylline stimulation in normal first-trimester and partial molar placenta responders, respectively. There was a gradual rise in secretion of hCG and hPL in both tissues in response to EGF during the culture period. Partial mole hCG responses were qualitatively less than those of normal placentas, but the differences were not statistically significant.

Secretion per cell was 0.70 ± 0.41 ng/ml hCG and 1.23 ± 0.21 ng/ml hPL, and the hPL/hCG ratio was 3.46 ± 1.89 (mean ± SEM) in partial moles. Normal

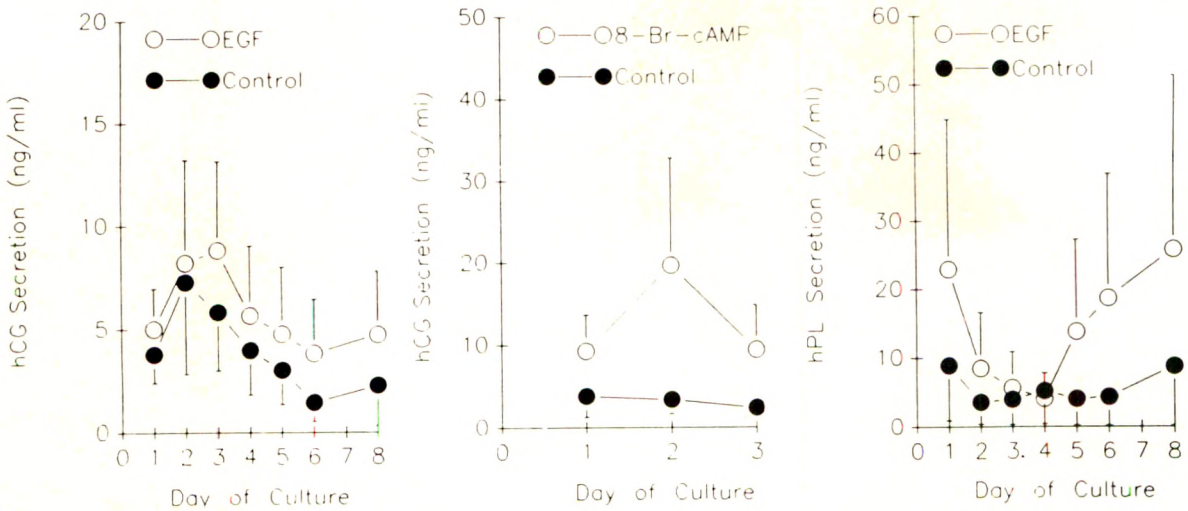


Fig. 4. Stimulation of hCG *(left panel)* and hPL *(right panel)* secretion by 10 ng/ml EGF or of hCG secretion *(middle panel)* by 1 mmol/L 8-bromo-cAMP/1 mmol/L theophylline of partial hydatidiform moles compared with controls. Mean ± SEM of responders appears in Table I. Responders were defined as having integrated hormone secretion over 7 culture days (EGF) or 3 culture days (8-bromo-cAMP/theophylline) that was significantly greater than control (see Material and methods). Each individual experiment (placenta) had significantly greater responses to stimuli than its own control. *Day of culture*, Culture day after EGF or 8-bromo-cAMP/theophylline was received.

first-trimester placenta secreted 3.59 ± 1.41 ng/ml hCG and 8.23 ± 3.26 ng/ml hPL, with an hPL/hCG ratio of 3.46 ± 0.55. The differences between partial moles and normal placenta were not significant.

The gestational age of all partial moles (14.3 ± 2.0 weeks, mean ± SEM, $n = 9$) was significantly greater than that of all normal placentas (10.0 ± 0.5 weeks, $n = 19$) by analysis of variance ($p < 0.01$). The two complete moles had gestational ages of 12.7 and 16.7 weeks. The age of one partial mole was unknown. The possible effect of gestational age on partial mole and normal placenta responses to 8-bromo-cAMP/theophylline and EGF stimulation was determined in two analyses, shown in Table II. Comparison of the gestational age of partial mole responders with partial mole nonresponders showed no significant difference (Table II). The same comparison showed no differences in the normal placenta group (Table II). The data were also analyzed by comparing partial mole responders and nonresponders with normal placenta responders and nonresponders, respectively (Table II). Although the gestational age of partial moles tended to be greater in all groups, only two comparisons showed statistical significance; 8-bromo-cAMP/theophylline responders were older in the partial mole group, and hPL nonresponders to EGF were also older in the partial mole group.

Comment

In the current study partial hydatidiform moles lacked response of hCG and hPL secretion to EGF stimulation and lacked a secretory response of hCG to 8-bromo-cAMP/theophylline. Partial moles thus showed a failure to develop differentiated function under EGF stimulation or to respond to 8-bromo-cAMP, a known secretagogue of hCG.[18] Complete moles showed a mixed response to these stimuli, but too few were available for statistical analysis. Few previous data in first-trimester placentas are available for comparison. Barnea et al.[17] showed that EGF induces rapid hCG release in a superfusion system and in static cultures but did not note morphologic differentiation. In term placentas we[14] and others[19] have found that EGF induces differentiated trophoblast function, that is, hCG and hPL secretion, and we have determined that the mechanism was due to differentiation of the cytotrophoblast into syncytium.[14] Thus most of the partial moles tested apparently lacked the ability to develop either a secretagogue response or possible morphologic differentiation after EGF exposure. It is unknown if this is due to EGF receptor deficiency or postreceptor events; further studies will be required to determine the mechanisms accounting for the defective response. In partial moles it is unknown why focal hyperplasia occurs. Because the hyperplasia is largely syncytial, the lack of an EGF response is consistent with uncontrolled proliferation. Differentiation ultimately occurs in partial moles; therefore factors other than EGF must be dominant in producing the hyperplasia. Such factors are currently unknown.

It is not clear why not all normal placentas responded to EGF or 8-bromo-cAMP/theophylline, but we pre-

Table II. Effect of gestational age of partial Moles and normal placentas on response to stimuli

	1 mmol/L 8-Bromo-cAMP/1 mmol/L theophylline			*10 ng/ml EGF*					
				hCG			*hPL*		
	No.	*Age* (mo)*	*p*	*No.*	*Age (mo)*	*p*	*n*	*Age*	*p*
Responders versus nonresponders									
Partial moles									
Responders	4	15.3 ± 2.7	NS	2	14.3 ± 6.3	NS	3	12.6 ± 4.1	NS
Nonresponders	4	14.4 ± 3.7		7	14.3 ± 2.2		6	15.1 ± 2.4	
Normal									
Responders	13	9.4 ± 0.6	NS	16	9.8 ± 0.6	NS	7	9.1 ± 0.8	NS
Nonresponders	3	10.8 ± 1.0		3	11.1 ± 1.1		11	10.7 ± 0.7	
Partial moles versus normal placentas									
Responders									
Normal placenta	13	9.4 ± 0.6	$p < 0.01$†	16	9.8 ± 0.6	NS	7	9.1 ± 0.8	NS
Partial mole	4	15.3 ± 2.7		2	14.3 ± 6.3		3	12.6 ± 4.1	
Nonresponders									
Normal placenta	3	10.8 ± 1.0	NS	3	10.8 ± 1.0	NS	11	10.7 ± 1.7	$p < 0.01$
Partial mole	3	12.7 ± 4.7		7	14.3 ± 2.2		6	15.1 ± 2.4	

NS, Not significant.
*Mean ± SEM.
†By analysis of variance.

sume this relates to tissue quality. Cell viability of preparations was not a factor because both normal and molar tissue had >95% viability by nigrosine dye exclusion.

In addition to a lack of EGF responsiveness, moles have demonstrated a variety of other hormonal abnormalities including low β-hCG/pregnancy-specific β_1-glycoprotein ratios in both partial and complete moles[10] and low prolactin (partial or complete mole not stated).[9] Complete moles and choriocarcinomas have high free α-subunit and free β-subunit production.[11, 12] The careful study by Berkowitz et al.[13] showed that both complete and partial moles had higher free α-subunit and higher free β-subunit levels than normal placentas. However, partial moles demonstrated higher free α-subunit levels in comparison with those of complete moles, and complete moles had higher free β-subunit levels than partial moles.

Consistently high hCG production has always been assumed to be proportional to increased tissue mass.[20] Our studies (Table I) indicate that the relative responsiveness of partial mole tissue to stimuli, and the hormone produced per cell, is not different from that of normal placenta, confirming this assumption. It is of interest that some studies have noted that hPL levels are lower in molar pregnancy,[6, 9] but we did not find this to be true in cultured cells, wherein the hPL/hCG ratios were similar. The reason for this discrepancy is not known.

We determined the effect of gestational age on the response to 8-bromo-cAMP/theophylline and EGF stimulation of hCG and hPL, because placentas of different ages may have responded differently (Table II). We observed that there was no difference in age between responders and nonresponders within each group separately (partial moles and normal placentas, Table II). We also analyzed the gestational age of responders compared to nonresponders (Table II). Partial moles tended in all comparisons to be older than normal placentas, but only two comparisons reached statistical significance. If age had affected the results, we would have expected responder moles–normal placentas to have different gestational ages than nonresponder moles–normal placentas. This was not observed. Together, these analyses indicate that the older gestational age of the partial moles could not account for their lack of response to stimuli. Consistent with this conclusion is the observation that the magnitude of response of first-trimester normal placentas to 8-bromo-cAMP/theophylline and EGF (Table I[17]) is similar to that seen in our previous studies of term placenta,[14, 18] implying no age-related alteration in trophoblast responsiveness to stimuli. Further studies would need to be conducted to specifically confirm this.

These studies also demonstrate that the antiserum for oncomodulin is useful as a positive cytotrophoblast marker, and GZ100 antiserum is useful to exclude syncytial contamination of cytotrophoblast preparations. Few such markers are available. It should be noted that the presence of Fc receptors on trophoblast[21] necessitates the use of $F(ab')_2$ fragments of polyclonal antisera

to ensure specificity. Additionally, it is of interest that partial and complete moles and normal placentas had the same pattern of expression of a small set of antigens, as tested in this study.

We conclude that partial moles have impaired differentiated function as represented by hCG and hPL secretion in response to stimulation by EGF and 8-bromo-cAMP/theophylline.

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Rapid growth of leiomyoma in patient receiving tamoxifen

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A 49-year-old patient with breast cancer had a leiomyoma that rapidly enlarged shortly after she started therapy with tamoxifen. Exploratory laparotomy was necessary to confirm the diagnosis. The rapid growth may have resulted from the estrogen agonist properties of tamoxifen or alternatively by ovarian stimulation resulting in endogenous estrogen production. (Am J Obstet Gynecol 1992;166:167-8.)

Key words: Tamoxifen, leiomyoma

Tamoxifen citrate is a nonsteroidal agent that has demonstrated estrogen agonist and antagonist properties.[1] Its antagonist properties make it a seemingly ideal drug for treatment of patients with breast cancers shown to be positive for estrogen receptors. Recently the recommendation has been made that all low-risk patients with breast cancer undergo adjuvant therapy.[2] This will result in a large number of perimenopausal and postmenopausal women being placed on a regimen of tamoxifen for 3 to 5 years. The gynecologic consequences of such widespread use are unknown because little attention has been given to this aspect of therapy. Previously, only high-risk patients or patients with recurrent disease were treated with tamoxifen. Now, low-risk patients undergoing therapy will live longer and will have a longer time in which side effects can develop. Because of the weak agonist properties of tamoxifen for estrogen-sensitive tissues, unexpected results may ensue from its usage. A patient is described to highlight a possible sensitivity for tamoxifen in a previously stable leiomyoma.

Case report

A 49-year-old woman, gravida 3, para 1, spontaneous abortions 2, with no living children, 5 feet 8½ inches tall, weighing 131 pounds, underwent a right modified radical mastectomy in August 1987 for removal of intraductal breast cancer. She received no further therapy. She was diagnosed in August 1988 with a left intraductal breast cancer and underwent a left modified radical mastectomy. Lymph nodes were negative, and tests for estrogen and progesterone receptors were positive. Because both tumors were estrogen receptor positive, in September 1988 she was started on tamoxifen, 10 mg twice daily, as adjuvant therapy, which the patient states she took as directed (assay is available). Her gynecologic history was unremarkable, and she was reported to have had a normal result of pelvic examination in July 1988 by the attending physician at the referring university hospital, before discovery of the second breast cancer. The patient had a computed tomographic scan of the abdomen performed before mastectomy, and at that time an abnormally thickened gastric wall was reported. It was otherwise normal. Three months after she started tamoxifen, a computed tomographic scan was performed at our institution for follow-up of the previously identified abnormality. The thickened gastric wall was normal; however, a large pelvic tumor, which previously had not been present, was discovered. On this scan she was noted to have a 10 × 15 cm, solid lower abdominal mass. On pelvic examination this filled the pelvis and was somewhat deviated to the right. It could not be distinguished from the uterus. Radioimmunoassay for pregnancy gave negative results, and an ultrasonographic examination revealed a probable leiomyoma with the possibility of a 4 cm cyst in one ovary. Menses had been regular before she started tamoxifen, with the last normal menstrual period on Sept. 4, 1988. She reported spotting for 1 day on Nov. 3, 1988.

At exploratory laparotomy the pelvis was noted to be markedly estrogen stimulated, similar to that seen with a term pregnancy. There was extensive venous engorgement. A total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. The remainder of the abdomen, including the stomach, was normal. The pathologic findings showed a 380 gm uterus with a benign leiomyoma 7 cm in diameter. The endometrium showed secretory endometrium. One ovary contained a corpus luteum cyst and the other contained a hemorrhagic corpus luteum. The patient had a normal postoperative course and was restarted on the previous dosage of tamoxifen. The patient is currently free of disease 26 months from the time of hysterectomy.

Comment

Tamoxifen competes with estradiol for receptor protein–binding sites. Hot flashes, vaginal discharge, and

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6/1/31298

irregular menses are the principal side effects of tamoxifen in the woman of reproductive age. All of these symptoms suggest the usual antiestrogenic effects attributed to tamoxifen. This drug does have agonist properties, however, and was originally developed as a fertility agent in the family of clomiphene citrate. In the case reported tamoxifen may have blocked the estrogen receptors in the hypothalamus, leading to an intense output of gonadotropins, which then led to intense ovarian stimulation and ovulation. This would explain the reported findings, consisting of secretory endometrium (evidence that ovulation occurred) and bilateral corpora lutea and enlargement of the leiomyoma. Alternatively, the pelvic organs may have responded to tamoxifen in an unexpected stimulatory fashion. It is possible that tamoxifen was bound to receptor sites on the leiomyoma, providing a greater stimulation than the perimenopausal estrogen level. This direct stimulation could then lead to rapid enlargement of the leiomyoma.

To our knowledge there are no previous reports in the literature concerning leiomyomas increasing in size under the stimulus of tamoxifen. However, it is known that tamoxifen has an apparent affinity for uptake by the endometrium and there is some selective uptake by the corpus luteum. Thus, in perimenopausal or postmenopausal patients in whom leiomyomas are expected to decrease in size after menopause and loss of estrogen, there may be a direct stimulatory effect by the action of tamoxifen. One report indicates no change in size of leiomyomas in six women receiving 20 mg tamoxifen per day.[3]

Surgical exploration was necessary in this patient because of the rapid growth of a new pelvic mass. The preoperative diagnosis included metastatic breast cancer, a primary ovarian tumor, leiomyosarcoma, or an enlarging leiomyoma, with this being the most likely diagnosis.

Until further cases are reported, it would seem prudent to advise all women who are to be treated with tamoxifen for breast malignancy to have a pelvic examination before such treatment. An initial follow-up examination could then be performed 3 to 6 months after tamoxifen is begun for those thought to have a leiomyoma and then annually to ensure that uterine size remains stable. Abnormal bleeding may also be a result of tamoxifen stimulation, and endometrial biopsy may be necessary in these patients should abnormal bleeding occur. Surgical exploration for enlarging leiomyoma will still be necessary to ensure that malignancy is not present, but the preoperative diagnoses will be less alarming.

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Transient severe unilateral and subsequent bilateral primary fetal hydrothorax with spontaneous resolution at 34 weeks' gestation associated with normal neonatal outcome

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Unilateral primary fetal hydrothorax with spontaneous resolution is a rare occurrence. We present a case in which severe unilateral primary fetal hydrothorax was visualized at 28 weeks' gestation. Two weeks later bilateral primary fetal hydrothorax was documented. Ultrasonographic follow-up demonstrated further dynamic changes in the fetal condition, with an isolated unilateral hydrothorax 1 week later and subsequent complete resolution at 34 weeks' gestation. Delivery resulted in a normal neonate with no signs of pulmonary hypoplasia. (Am J Obstet Gynecol 1992;166:169-70.)

Key words: Unilateral fetal hydrothorax, spontaneous resolution

Fetal pleural effusions are either primary or secondary. Primary effusions are usually chylous, as opposed to secondary collections, which are more often associated with overall fluid retention seen with nonimmune fetal hydrops.[1] The incidence of primary fetal hydrothorax is estimated at one case per 15,000 pregnancies.[1] In the past only three cases of primary fetal hydrothorax with spontaneous resolution have been reported.[1] The following case describes a fetus with severe unilateral pleural effusion at 28 weeks' gestation, which then became bilateral and resolved spontaneously at 34 weeks with normal neonatal outcome.

Case report

A 20-year-old woman, gravida 2, para 0, was seen for her first antenatal visit at 21 weeks' gestation when she presented with spotting. At that time fundal height was S + 21 cm. Laboratory results were: hematocrit 32%, blood type B, Rh positive, negative antibody screen, and Venereal Disease Research Laboratory test and human immunodeficiency virus negative. Ultrasonographic examination revealed a singleton fetus with normal fetal anatomy and a composite gestational age of 21 weeks 4 days. Placental location was posterior, midheight, with no signs of abruptio placentae. At 28 weeks 3 days, repeat ultrasonography performed to document fetal growth disclosed a massive right unilateral primary fetal hydrothorax (Fig. 1). The ratio of lung span to hemithorax diameter was 0.46.[2] No signs of fetal hydrops were noted and a complete fetal echocardiogram was normal, apart from a mild pericardial effusion within normal limits (Fig. 1, *B*). Genetic amniocentesis was performed and subsequently showed a normal 46,XX karyotype. Further workup for nonimmune hydrops, which included maternal testing for α-thalassemia, glucose-6-phosphatase dehydrogenase deficiency, and a Kleihauer-Betke test, failed to disclose a possible cause of the fetal hydrothorax. Venereal Disease Research Laboratory, TORCH, (toxoplasmosis, rubella, cytomegalovirus, and herpes simplex) and parvovirus titers were negative. Repeated detailed ultrasonography did not disclose any further fetal anomalies. Follow-up ultrasonography at 30 weeks 5 days' gestation demonstrated bilateral hydrothorax (Fig. 2, *A*), again without any other signs of fetal hydrops. Because of this dynamic development and anticipation that preterm delivery might be indicated if fetal hydrops were to appear, 12.5 mg intramuscular betamethasone was administered twice to enhance fetal pulmonary maturation, and weekly ultrasonographic follow-up was initiated. At 31 weeks 2 days' gestation the hydrothorax was notable only on the right. This accumulation of fluid markedly decreased at 32 weeks 4 days and at 34 weeks 1 day had completely resolved (Fig. 2, *B*). Subsequent weekly ultrasonographic studies remained unchanged. At 36 weeks induction of labor was performed because of severe oligohydramnios. The patient was delivered vaginally of a female infant weighing 2440 gm with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. Umbilical artery blood gas values were pH 7.35, P_{O_2} 25 mm Hg, P_{CO_2} 43 mg, oxygen saturation 51%, and base excess −2 mEq/L. Neonatal examination revealed a completely healthy infant with normal chest roentgenogram and electrocardiogram. Both mother and infant were discharged in good health on postpartum day 3.

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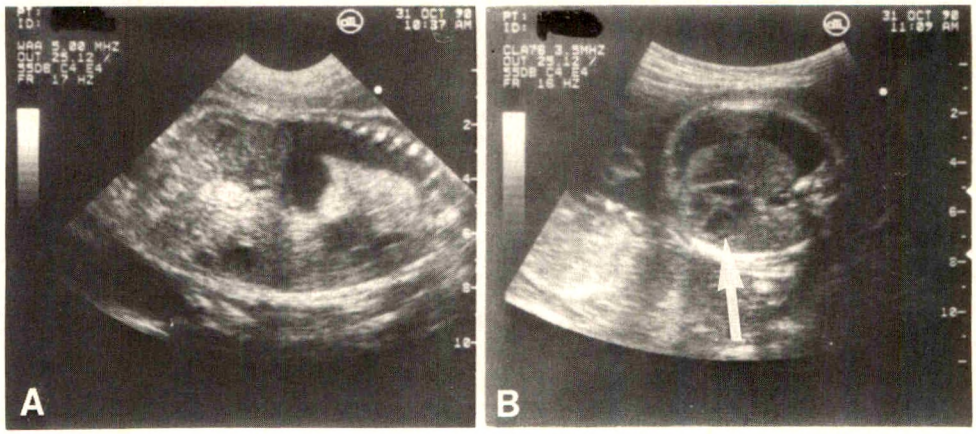


Fig. 1. A, Longitudinal scan of right fetal thorax at 28 weeks 3 days' gestation. Note large primary fetal hydrothorax and compressed right fetal lung. **B,** Transverse scan. *Arrow,* Mild pericardial effusion.

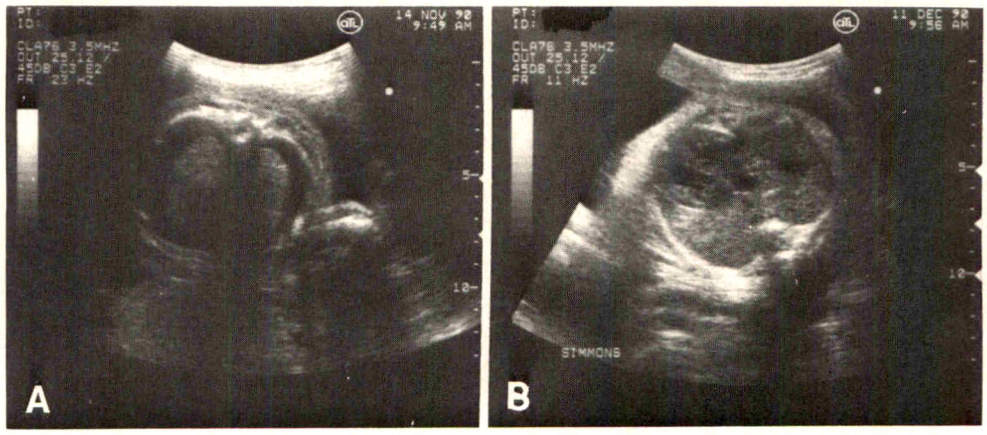


Fig. 2. A, Transverse scan at 30 weeks 5 days' gestation. Note bilateral fetal hydrothorax. **B,** Transverse scan at 34 weeks' gestation. Note absence of fetal hydrothorax bilateral.

Comment

Large persistent pleural effusions may prevent normal lung development. Crucial factors in the development of pulmonary hypoplasia are time of onset, severity, and duration of pleural effusions.[1] Our case is unique in that serial ultrasonographic examinations documented late midtrimester, severe, unilateral primary fetal hydrothorax with later bilateral involvement finally resolving at 34 weeks. In the absence of fetal structural or chromosomal anomalies or immune or nonimmune fetal hydrops, the cause of transient pleural effusions is generally unknown. However, one may speculate that intermittent mild fetal cardiac failure may have precipitated this event. Undetected causes could include brief fetal cardiac arrhythmias, transient fetal hypoalbuminemia with associated low colloid osmotic pressure, or an undiagnosed fetal infection. In the presence of intact membranes and the subsequent delivery of an appropriate-for-gestational-age, nonhypoxic, nonacidotic infant, the cause of the oligohydramnios is unclear. It is doubtful whether the oligohydramnios and pleural effusions were related.

Castillo et al.[2] recently noted a lung span–to–hemithorax diameter ratio of 0.44 to 0.77 (mean 0.60) to be uniformly associated with pulmonary hypoplasia. Our case stresses the inability to predict pulmonary hypoplasia on the basis of these ultrasonographic indices alone in cases in which dynamic amounts of fluid collection and lung compression occur. Clearly emphasized is the importance of two main pathophysiologic factors underlying pulmonary hypoplasia, namely, the time of onset and the duration of this insult. We believe that the late onset, beyond 20 weeks' gestation, and limited duration of the fetal hydrothorax in our case contributed to the successful outcome.

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Meconium aspiration syndrome: Reflections on a murky subject

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Meconium-stained amniotic fluid occurs in approximately 12% of live births. In approximately one third of these infants meconium is present below the vocal cords. However, meconium aspiration syndrome develops in only 2 of every 1000 live-born infants. Ninety-five percent of infants with inhaled meconium clear the lungs spontaneously. Recent investigations have suggested that a reexamination of our assumptions about the etiology of meconium aspiration syndrome is in order. Several authors have provided evidence that support the hypothesis that it is not the inhaled meconium which produces the primary pathologic condition of meconium aspiration syndrome but rather it is fetal asphyxia that is the etiologic agent. Asphyxia in utero produces pulmonary vasospasm and hyperreactivity of the pulmonary vessels. With severe asphyxia the fetal lungs undergo pulmonary vascular damage with pulmonary hypertension. The damaged lungs are then unable to clear the meconium. In the most severe cases there is right-to-left shunting and persistent fetal circulation with subsequent fetal death. The incidence of meconium aspiration may thus be essentially unaffected by current obstetric and pediatric interventions at birth. For the asphyxiated or distressed infant we recommend suctioning at birth and tracheal intubation. In the healthy fetus observation may be sufficient. (Am J Obstet Gynecol 1992;166:171-83.)

Key words: Meconium aspiration, asphyxia, postdates

This review questions three long-standing assumptions about meconium. The first is that the presence of meconium in the amniotic fluid is an independent marker of fetal distress. The second is that meconium aspiration primarily occurs when the fetus gasps at birth or in utero, in response to severe asphyxia. The third is that meconium aspiration syndrome is a result of the deleterious effect of the inhaled meconium on the neonatal lungs.

Generations of clinicians have cared for infants dying of respiratory causes after being born through meconium-stained fluid. Our assumptions about meconium aspiration are based on a cause-and-effect relationship derived from such patients. These assumptions link the inhalation of meconium and subsequent severe respiratory distress. When "pea soup" meconium is noted during labor and the nasopharynx and trachea are suctioned, the clinician feels relief and satisfaction if the infant subsequently does well. However, a review of recent reports questions whether the respiratory disease of meconium aspiration syndrome is caused simply by the aspiration of meconium. The clinician who has appropriately managed a case of an infant with meconium has most likely obtained a favorable outcome, not by suctioning the nasopharynx but by avoiding perinatal asphyxia.

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Meconium passage

Several studies have examined meconium passage. In the largest series meconium was found in the amniotic fluid of approximately 21,500 of 177,000 live births.[1] The Collaborative Perinatal Project documented a slightly lower incidence of 10.3%,[2] because stained membranes rather than discolored amniotic fluid were counted. Meconium staining of the amniotic membranes begins 1 to 3 hours after the fetus passes meconium.[3] Various series (Table I) have noted incidences between 7% and 22% of live births.[1, 2, 4-20] Meconium passage is rare before 38 weeks but occurs more than 30% of the time in pregnancies >42 weeks' gestation.[6-8, 20-24] Green discoloration of amniotic fluid is found in 1% to 3% of second-trimester amniocentesis

Table I. Population studies evaluating meconium

Author	No. of live-born infants	Patients passing meconium (%)	Infants with meconium aspiration syndrome			
			Proportion of total infants with meconium (%)	Proportion of all live births (%)	Severe disease (%)	Mortality (%)
Wiswell et al.[1]	176,790	12.2	5.4	0.7	30	4.2
Fujikura and Klionsky[2]	42,490	10.3*	–†	–	–	–
Johnson[4]	1,514	18.0	–	–	–	–
Coltart et al.[5]	38,395	–	–	0.2	32	8.3
Meis et al.[6]	2,923	22.0	2.1	0.4	–	7.7
Usher et al.[7]	7,663‡	19.5	1.8	0.4	0	0
Benny et al.[8]	6,958	10.9	8.7	0.9	32	6.1
Ostrea and Naqvi[9]	5,025	7.0	–	–	–	–
Sepkowitz[10]	582	14.4	–	–	–	–
Rossi et al.[11]	1,183	22.0	8.6	1.9	32	18
Carson et al.[12]	11,586	13.8	1.6	0.2	27	19 (0§)
Hageman et al.[13]	3,230	14.4	3.0	0.4	43	0
Locus et al.[14]	2,874	12.0	1.7	0.2	33	0
Falciglia[15]	25,499	15.4	1.4	0.2	27	46 (12.5§)
Desmond et al.[16]	3,967	10.9	–	–	–	–
Linder et al.[17]	6,500	10.4	–	–	–	–
Gregory et al.[18]	1,000	8.8	18.0	1.6	0	0
Davis[19]	9,299	15.0	2.1	0.3	—	40

*Meconium-stained membranes.

†Indicates data not reported.

‡Study population: Live-born infants with ultrasonographic confirmation of gestational age after 38 completed weeks.

§After institution of combined suction.

procedures.[25-27] This discolored fluid has been noted to contain multiple hemoglobin breakdown products.[25, 26] Table II summarizes series that have examined meconium passage as a function of gestational age.

The hormonal control of fetal meconium passage is maturationally dependent.[28-30] Motilin, an intestinal peptide, is a hormone responsible for bowel peristalsis and defecation.[29] Umbilical cord motilin levels are higher in infants who have passed meconium compared with those in infants with clear amniotic fluid, and motilin levels are lower in premature infants than in term and postterm infants.[29-31]

The neural control of meconium passage is also dependent on gestational age, because maturation and myelination of the neural plexus of the gastrointestional tract progress throughout gestation.[28] As the nervous system matures, parasympathetic stimuli may be propagated and initiate defecation. Under laboratory conditions, when subjected to umbilical cord compression or fetal hypoxia, many near-term fetal animals pass meconium.[32, 33] Thus vagal stimulation, such as from umbilical cord or head compression, will initiate meconium passage only after the innervation of the fetal intestinal tract has matured.

Meconium and fetal distress

The first clinical assumption that many authors have questioned is whether meconium is an independent marker of fetal distress. Obstetrics texts throughout the seventeenth, eighteenth, and nineteenth centuries reported meconium passage as a sign of fetal death or impending death.[34] Several modern studies have examined data collected around the time of birth to correlate meconium passage and fetal distress. Such data must be viewed cautiously because the association of the events of labor and particularly of delivery does not necessarily represent the intrauterine environment at the time of meconium passage. For example, analysis of Apgar scores to correlate distress with meconium-stained fluid may be misleading, because Apgar scores are affected by tracheal intubation. Blood gas analyses may be more accurate indicators of fetal well-being. For meconium to be an independent marker of fetal distress, infants with meconium staining would have adverse perinatal outcomes without other evidence of fetal compromise. This has not been found to be the case.

Table III summarizes studies that have examined the relationship between meconium and fetal distress. In general, when normal fetal heart rate (FHR) patterns are found with meconium-stained fluid, the neonatal outcome is similar to that of neonates with clear fluid.[11, 16, 24, 35-41] Similarly, when infants with meconium have antepartum signs of distress, such as fetal tachycardia, decreased FHR variability, and the absence of accelerations, the neonatal outcomes are similar to those of non-meconium-stained infants with similar FHR abnormalities.[11, 36, 38, 42, 43] Among infants in the Collaborative Perinatal Project the incidence of meco-

nium in the amniotic fluid was similar in asphyxiated and nonasphyxiated infants.[41] Thus meconium is not an independent marker of fetal distress but rather a result of an event, such as cord compression, and often an environment, such as decreased amniotic fluid, that may predispose to or be a consequence of fetal compromise. Some fetuses with meconium-stained fluid will be compromised, but most will not. As Miller et al.[35] have stated, "the presence of meconium in the amniotic fluid without signs of fetal asphyxia . . . is not a sign of fetal distress . . . ."

Meconium aspiration

The second assumption is that meconium aspiration is primarily a consequence of a fetal gasping at birth. Meconium aspiration may be defined as the presence of meconium below the vocal cords (as opposed to meconium in the oropharnyx). Meconium is found below the vocal cords in 11% to 58% (mean 35%) of live births with meconium-stained amniotic fluid, approximately 4% of all live births (Table IV).[11, 13, 15, 18, 40, 43-45] Meconium may pass through the trachea and into the lungs through two related mechanisms, gasping and deep breathing.

Fetal gasping is a normal response to asphyxia. The asphyxia may vary from a mild increase in carbon dioxide to severe acidemia and may represent temporary umbilical cord occlusion or profound metabolic acidosis.[32, 33, 46] Dawes et al.[32] induced meconium aspiration in fetal lambs with umbilical cord occlusion. The fetal lambs passed meconium, gasped, and inhaled the meconium into their lungs. When the occlusion was relieved, amniotic fluid and meconium passed out of the fetal lungs. Gasping has been induced in rhesus monkeys and baboons.[47, 48] Block et al.[47] produced meconium aspiration after placing meconium in amniotic fluid of pregnant baboons and then occluding the maternal aorta.

Human gasping has been described in high-risk fetuses 24 to 72 hours before intrauterine death.[49] Amniotic fluid, cellular debris, and meconium may be found in the lungs of fetuses who have died in utero of asphyxia.[50, 51] Autopsies of severely asphyxiated preterm stillborn fetuses consistently demonstrate amniotic fluid and debris in the fetal lung parenchyma, but meconium is rarely found.[51, 52] This is because meconium is seldom present in the amniotic fluid of preterm infants.

Fetuses also aspirate meconium and amniotic fluid by deep (irregular) breaths in utero. The net flow of fluid is out of the fetal lungs into the amniotic cavity with the shallow, regular thoracic motion that constitutes 90% of fetal "respirations".[32, 33, 49, 53] Shallow, regular "breathing" may demonstrate some forward and backward flow. However, Dawes et al.[32] found repetitive

Table II. Meconium passage as function of gestational age

Series	*Infants with meconium passage (%)*
Usher et al.[7]	
39-40 wk	15.3
41 wk	27.0
≥42 wk	31.5
Ostrea and Naqvi[9]	
<38 wk	4.3
39-42 wk	6.0
≥42 wk	52.0
Eden et al.[20]	
39 wk	13.5
40 wk	19.4
42 wk	25.5
>42 wk	28.9
Steer et al.[24]	
<36 wk	3.0
36-39 wk	13.0
40-41 wk	19.0
≥42 wk	23.0

periods in which the fetus made "relatively deep inspiratory efforts" in unasphyxiated animals. These deep inspiratory efforts were seen more frequently as gestation advanced and represented up to 10% of all fetal "breathing." This normal deep breathing may account for some of the meconium that is inhaled in utero. Thus meconium below the vocal cords may be related to the concentration of meconium, as well as to the duration of meconium exposure.

In nonasphyxiated, nonhypoxic human fetuses, regular passage of amniotic fluid deep into the fetal lung is well documented. Injection of radiopaque contrast medium into amniotic fluid has produced images of the fetal lungs in utero.[38] Duenhoelter and Pritchard,[54] injected chromium 51–labeled red blood cells intraamniotically. The red blood cells were found only deep in the fetal lung parenchyma and in the stomach in both abortuses and fetal rhesus monkeys that were exposed to the red blood cells for 24 hours. To show that the chromium-labeled cells were not inhaled immediately before delivery, iron 59–labeled red blood cells were also injected into the amniotic fluid shortly before delivery. There were many chromium-labeled cells but few iron-labeled cells deep in the fetal lungs. In their studies Duenhoelter and Pritchard showed that human fetuses as well as rhesus monkey fetuses, inhale approximately 200 ml/kg/24 hours.

Fetal hypercarbia will increase the incidence of deep breathing.[53] An increase in the P_{CO_2} of as little as 5 mm Hg may induce increased deep breathing.[55] This level of hypercarbia without asphyxia can occur from transient umbilical cord compression.[32, 48, 53] Martin et al.[48] demonstrated deep breaths concurrently with variable

Table III. Studies evaluating fetal distress by comparing meconium-stained infants and controls with clear fluid

Series	*No. of subjects*	*Variables matched in comparison group*	*Other variables*	*Outcome: Meconium vs control*
Desmond et al.[16] (1957)	407	Infant birth weight	Neonatal mortality	3.4% vs 1.5%
			Intrapartum complications	(20.4 vs 21.9)
Meis et al.[6] (1978)	614	Maternal age and parity	5 min Apgar scores	Lower with thick meconium
			Perinatal mortality	
			Heart rate changes	More fetal tachycardia and less variability with meconium
Miller et al.[35] (1975)	366	Consecutive deliveries	Variable and late decelerations	No difference
			Mean 5 min Apgar scores	8.8 vs 8.4
			% Infants with late decelerations and pH < 7.25; low 5 min Apgar scores	No difference
Bochner et al.[36] (1987)	839	Postdate pregnancies at antepartum testing unit	5 min Apgar scores <7	No difference
			Mortality	
Steer et al.[24] (1989)	790	Consecutive deliveries	Umbilical artery pH	No difference
			Umbilical artery base deficit	
			5 min Apgar score	7.17 vs 7.22
			Abnormal first-stage FHRs	Umbilical artery pH
			Abnormal second-stage FHRs	No difference in pH
Abramovici et al.[37] (1974)	160	Age, parity, cervical examination when meconium noted	Apgar scores	No difference
			Mortality	
Krebs et al.[38] (1980)	1956	Consecutively monitored patients	% Abnormal FHR score first 30 min monitored	1.8% vs 1.1%*
			% Abnormal FHR score last 30 min monitored	7.4% vs 3.2%*
			% Low 5 min Apgar score with abnormal FHR pattern	No difference
			% Low 5 min Apgar score with normal FHR pattern	No difference

*Difference significant at $p < 0.01$.

decelerations in rhesus monkeys. Other investigators[32, 33, 53] have produced gasping with umbilical cord occlusion. Such umbilical cord occlusion may not be sufficiently prolonged to induce fetal distress, manifested as acidemia, yet may increase P_{CO_2}, which results in deep respirations that draw meconium into the lungs. Although hypoxia decreases breathing motion, hypoxia and hypercarbia act synergistically to stimulate gasping.[56]

Several investigations[11, 13, 15, 43, 44] have examined the status of infants with meconium below the vocal cords (Table IV). The finding of meconium below the cord is rarely a consequence of severe fetal compromise. However, there is a subgroup of infants with meconium below the cords who will be severely distressed. Dooley et al.[44] analyzed 272 births of infants with meconium-stained fluid. Meconium was found below the cords in 21% of these infants. When the groups with and without meconium below the cords were compared, there was no difference in gestational age, birth weight, umbilical cord pH, P_{CO_2}, P_{O_2}, base deficit, percent of infants with pH <7.2, or base deficit >5 mEq/ml. In a study by Yeomans et al.[43] of meconium-stained infants, among a group of infants with meconium below the vocal cords, 69% had an umbilical artery pH >7.19. Eleven percent of infants in the study of Yeomans et al. had an umbilical cord pH <7.15, corresponding to the infants who may aspirate as a secondary result of severe asphyxic stress. Five percent of infants had low umbilical cord pH and did not have meconium below the vocal cords.

In a study of 238 births with meconium-stained amniotic fluid, Rossi et al.[11] found meconium below the cords more commonly in the presence of thick meco-

Table IV. Studies evaluating meconium aspiration

Series	No. of meconium-stained infants	Proportion with meconium below vocal cords (%)	Meconium below vocal cords and markers of distress	Infants with meconium aspiration syndrome		Combined suction*
				Proportion of total infants in study (%)	Proportion of infants with meconium below vocal cords	
Yeomans et al.[43]	323	23	pH >7.19 in 69%	1.9	8	Yes
Mitchell et al.[40]	53†	–	pH <7.25 in 100%	17	—	Yes
Rossi et al.[11]	238	37	Mean pH‡	8.6	12§	Yes
Dooley et al.[44]	272	21	Mean umbilical pH‡ Base deficit‡ Incidence of pH <7.2‡	0.4	22	Yes
Hageman et al.[13]	464	31	Mean pH 7.23	3	10	Yes‖
Burke-Strickland and Edwards[45]	101	50	—	—	—	Yes
Falciglia[15]	755	37	—	2	6	Yes
Gregory et al.[18]	80	58	—	20	35	Yes

*By obstetrician and pediatrician.

†Thick meconium.

‡No different than matched infants without meconium below the cords.

§A total of 47% of infants with meconium aspiration syndrome did not have meconium below the vocal cords.

‖In 92% of patients.

nium. This finding agrees with the theory that the greater the concentration of meconium in the amniotic fluid, the more meconium will pass transtracheally.

Timing of meconium aspiration

In the 1970s through the late 1980s, many authors[12, 18, 22, 35] felt that meconium was aspirated with the infant's first breath. This concept arose from an influential experiment by Gooding et al.[57] in which puppies could not be induced to aspirate meconium until it was injected below the vocal cords before the puppies' first breath.

Carson et al.[12] advocated oral DeLee tube suction by the obstetrician followed by tracheal intubation and suction of meconium by the pediatrician, all before an infant's first breath. In a retrospective review they found five deaths in 18 cases of meconium aspiration syndrome without combined suction and no deaths among seven cases of meconium aspiration syndrome after the institution of the combined suction protocol. Their conclusion was that because aspiration occurred with the first breath, clearing the airway decreased the severity of meconium aspiration syndrome. When other investigations confirmed the findings of Carson et al., the concept of meconium aspiration occurring with the infant's first breath was established.[18, 22, 45]

Within a few years of the work of Carson et al., several reports emerged that questioned this concept of meconium aspiration. Meconium was found below the vocal cords in fetuses dying in utero and in infants before their first breath. Severe disease developed in spite of suction at delivery (Table IV).[7, 11, 13, 15, 18, 19, 40, 43-45, 50, 51] Falciglia,[15] in reviewing a series of 4896 deliveries, concluded: "Our findings suggest that meconium aspiration is predominantly an intrauterine event." The aspiration of amniotic fluid is not only an intrauterine event but also an antepartum process. In the fetus with oligohydramnios and cord compressions, meconium aspiration will continue during labor, with continued fetal gasping.

Meconium aspiration syndrome

The third assumption that recent investigations have focused on is that the pathologic condition of meconium aspiration syndrome is primarily a result of the injurious effects of meconium on the fetal and neonatal lungs. A review of the clinical findings in meconium aspiration syndrome suggests that parenchymal lung damage or airway obstruction from inspired meconium is not the primary cause of the pathophysiologic condition.

Meconium aspiration syndrome represents a wide spectrum of clinical disease. Mild transient distress in the setting of meconium-stained amniotic fluid, without the need for treatment, has been labeled "subclinical meconium aspiration syndrome."[5] Respiratory distress

with minimal oxygen requirements (<40%) for <48 hours is considered mild disease.[11] Respiratory distress lasting for >48 hours with an oxygen requirement necessitating mechanical ventilation is usually defined as severe disease.[11] Meconium aspiration syndrome is fatal in up to 40% of cases (Table I); the percentage varies with the extent to which mild aspiration syndrome is included in the diagnosis. Almost all disease is manifest within the first few hours of birth, and severe disease is often manifest from the time of delivery. Fatal meconium aspiration syndrome may occur within minutes of birth.[19, 50, 58, 59]

The radiographic findings of the chest x-ray film are specific and diagnostic for meconium aspiration syndrome.[60] These findings include coarse and patchy areas of decreased aeration, areas of widespread consolidation or infiltrates, and occasional areas of increased aeration.[60] Small pleural effusions occur in up to one third of infants. Air leaks represented by pneumothoraces and pneumomediastinum occur in up to one quarter of all infants.[1, 11, 18, 22, 60-63] When sequential radiographs are obtained during the course of the disease, labeled meconium can be seen to migrate into the lung parenchyma over the first 4 hours after birth.[57, 60, 64] Areas of diminished aeration clear within 24 hours, and 80% of all x-ray films are clear within 48 hours.

Forty percent to 73% of infants born with meconium-stained fluid have positive chest x-ray findings, although fewer than half of these infants show signs of respiratory distress.[18, 45, 60] In addition, the severity of x-ray findings may not always correlate with the clinical disease. Some infants may experience severe disease with minimal x-ray findings, while other infants have remarkable x-ray findings without clinical disease.[18, 65]

Hypoxia is the primary clinical problem for infants with meconium aspiration syndrome. The hypoxia may be mild or profound. Profound hypoxia is usually secondary to right-to-left shunting.[62, 63, 65-69] Measurement of oxygen saturation during ventilation with 100% oxygen demonstrates shunting of varying degrees.[66, 69] In one study shunts from 27% to 73% were documented.[65] Severe meconium aspiration syndrome is associated with pulmonary hypertension and persistent fetal circulation, with a patent ductus arteriosus and patent foramen ovale.[1, 6-8, 11, 13, 15, 19, 44, 50, 51, 52, 59, 61-69] Neonatal cardiac catheterization in infants with meconium aspiration syndrome has demonstrated high right ventricular and pulmonary artery pressures equal to or exceeding systemic arterial pressures.[66]

Autopsy findings include meconium, vernix, fetal squamous cells, and cellular debris at different levels in the lungs from the small airways deep into the alveoli. Meconium may obstruct some airways.[19, 51, 58, 59, 70] Occasionally, minimal meconium or no meconium will be found in the lungs, although the clinical disease mimics classic meconium aspiration syndrome.[19, 71] The inflammatory response to meconium may appear as consolidation and pneumonia with polymorphonuclear leukocytes, macrophages, and edema. However, large quantities of meconium with wide distribution are often found deep in the lungs without histologic signs of inflammation.[70] Hyaline membranes are frequently seen in infants surviving for >24 hours.[70, 72] In one autopsy series of postmature infants, 18 of 21 infants had hyaline membrane disease and 17 of these 18 died of meconium aspiration syndrome.[72]

Pathologic conditions in the pulmonary vasculature of infants dying of meconium aspiration syndrome include diffuse and severe pulmonary hemorrhage and necrosis of pulmonary vessels and pulmonary parenchyma.[19, 58, 59, 71, 73] Platelet-rich microthrombi in small arterioles have also been noted.[74] Muscularization in the distal pulmonary vasculature has been found in severe meconium aspiration syndrome.[70, 75-77] Ordinarily, muscular pulmonary vessels course together with the airways and extend to the level of the terminal bronchioles.[63, 69, 75-77] Less than 20% of pulmonary vessels traversing along the respiratory bronchioles have identifiable smooth muscle. However, many infants dying of meconium aspiration syndrome, whose clinical course is characterized by persistent pulmonary hypertension, have muscularity of the small vessels extending to the distal arterioles and into the vessels surrounding the alveoli.[63, 67, 70, 75-77] Perlman et al.[70] found muscularization not only in stillborn infants and neonates dying of meconium aspiration but also in stillborn infants dying of abruptio placentae. Excessive muscularization takes 3 to 7 days to develop in utero and has been reproduced in animals by fetal exposure to hypoxia.[77-81]

The theory of meconium aspiration syndrome in question is that the pathologic characteristics of the disease are primarily a result of the effects of meconium on the lung parenchyma. This theory does not account for the following: (1) 35% of infants born with meconium in the amniotic fluid will have meconium in the lungs, with up to 55% of all meconium-stained infants having abnormal chest x-ray findings, yet respiratory distress develops in only 5% to 10% of infants with meconium below the vocal cords; (2) some infants die in utero or within the first hour of life, with histologic evidence of meconium aspiration; (3) clinical disease may occur in some cases without signs of inflammation, occasionally with no meconium at all found below the vocal cords.

Etiology of meconium aspiration syndrome

Recent investigations have advanced the hypothesis that the respiratory distress and hypoxia of meconium

aspiration syndrome are initiated by pulmonary vascular disease. The pathophysiologic changes are vascular spasm and vascular hyperreactivity of the pulmonary vessels. In mild cases the vascular disease is a transient, reversible hyperreactivity manifest as brief episodes of desaturation.[61, 62, 68] In the most severe cases of meconium aspiration syndrome, the pulmonary vascular disease is a fixed pulmonary hypertension with persistent fetal circulation and pulmonary vascular necrosis and hemorrhage.[63, 65, 67, 72] The normal transition from fetal to adult circulation, from pulmonary vasoconstriction to pulmonary vasodilatation, is profoundly disrupted in infants with severe meconium aspiration syndrome.[63, 77, 78, 81] The cause of the pulmonary vascular spasm, is fetal hypoxia and asphyxia.[61, 62, 63, 66, 70, 77, 82, 83] Meconium inhaled below the vocal cords in an unasphyxiated infant produces only a mild, benign, self-limited respiratory challenge that is asymptomatic >90% of the time.[11, 15, 44] Meconium aspiration syndrome is a multifaceted disease. Antenatal asphyxia is the primary problem that injures the lungs. Meconium may compound the insult by causing localized areas of pulmonary hypoxia, as well as a chemical pneumonitis. Infection also may contribute to the pathophysiologic condition by further inducing pulmonary vasospasm and by injuring the alveoli.

The normal fetal pulmonary vasculature remains constricted until birth. With the transition to an extrauterine environment, the sudden increase in the size of the pulmonary vascular bed causes an abrupt fall in pulmonary arterial pressure.[67, 78] Pulmonary circulation increases within 1 minute from 10% of the cardiac output to 50%.[67, 80] The muscular arteries in the proximal pulmonary arterial circulation undergo a shrinkage in wall thickness and contractile cells over the next 3 to 4 days. Although the complete mechanism of pulmonary vasorelaxation is unknown, increasing P_{O_2} and expansion and inflation of the lungs at birth, as well as humoral factors, are involved.[67-69, 75, 77, 78, 83, 84] In contrast, hypoxia produces vasoconstriction in both the fetal and the neonatal pulmonary circulation.[67-69, 77, 78, 83]

In the newborn the pulmonary vascular response to hypoxia is dependent on both the age of the infant and previous hypoxic exposures. In the infant who has progressed beyond the first week and in the adult, hypoxia produces increased pulmonary artery pressures.[85] However, the increase in pulmonary artery pressure is always less than the systemic arterial pressures. In contrast, in the fetus and very young newborn, hypoxia can produce a rise in pulmonary artery pressure that is greater than the systemic artery pressure.[85] Clinically, such an increase in pressure can produce a return to the fetal circulation, with shunting of blood from the right side of the circulation to the left side with a precipitate decrease in oxygen saturation. In this way the newborn response to hypoxia is different than that of older infants or adults.[85, 86]

The critical factor that influences the response of a newborn to hypoxia is the prior exposure of the fetus to a low P_{O_2}. Hypoxia and asphyxia in utero produce a pulmonary vasoreactivity that lasts well into the newborn period.[61, 62, 75-77] This pulmonary vasoreactivity may cause marked increases in pulmonary artery pressures in response to only minimal changes in oxygen in the newborn. This pulmonary vascular spasm may be "set off" by changes in temperature, stimulation, or any irritation that results in a change in systemic arterial pressures (as well as by hypoxia). Slight hypoxia may set off a vicious cycle in which resulting vasospasm causes worse hypoxia and additional vasospasm.[61, 62, 66-72, 82, 83]

The severity of the vasospasm is dependent on the extent of the fetal hypoxic insult.[68, 78, 82, 83] If the fetal asphyxia was brief and mild, the neonatal pulmonary vasospasm may result in transient shallow declines in oxygen saturation and respond readily to oxygen therapy.[67, 78, 82] If the asphyxia in utero was severe, the pulmonary vasoreactivity may be manifest as a fixed pulmonary hypertension, with extensive right-to-left shunting and histologic findings of muscularization of the most distal pulmonary arterioles.[61-63, 66-73] It is this pulmonary vasohyperreactivity that initiates the pathologic condition of meconium aspiration syndrome. Severe fetal hypoxic insults are rare; thus severe meconium aspiration syndrome is rare, occurring in <2% of infants who pass meconium (approximately 2/1000 live-born infants).[1]

Several investigators have demonstrated the cause-and-effect relationship between fetal asphyxia and neonatal pulmonary vascular disease.[87-92] Drummond and Bissonnette,[87] using placental embolization, produced both pulmonary hypertension and an exaggerated response to hypoxia in newborn lambs. Gersony et al.[88] produced pulmonary hypertension, persistent fetal circulation, and pulmonary vascular spasm in newborn lambs by creating maternal hypotension with aortic occlusion of the pregnant ewes and subsequent decreased placental blood flow. These investigators reproduced in newborn lambs the downward spiral of hypoxia, pulmonary vascular spasm, right-to-left shunt, worsening hypoxia, worsening pulmonary hypertension, and neonatal death seen in meconium aspiration syndrome.[88]

Excess muscularization is the most severe form of pulmonary vascular disease in meconium aspiration syndrome. Experimentally, fetal exposure to hypoxia produces excessive muscularity in pulmonary vessels of rats in 3 to 7 days.[79-81] Newborn pulmonary hypertension may also be produced by the induction of fetal systemic hypertension.[91] In sheep, prolonged fetal arterial hypertension produced by ligation of one renal

artery led to fixed pulmonary hypertension and distal pulmonary vessel muscularization.[91]

One of the most important experiments pertaining to meconium aspiration in the past three decades was performed by Jovanovic and Nguyen,[93] who illustrated the relationship between severe in utero hypoxia and meconium aspiration syndrome.[93] Fetal guinea pigs were exposed to either a pH of 7.10 for 1- and 2-hour periods before delivery or to a pH of 7.10 for 1 hour per day for 2 weeks before delivery, or they were delivered without hypoxic exposure. At the time of delivery, before the first breath, the tracheas of the guinea pig pups were injected with either amniotic fluid or meconium. After 24 hours, in the unasphyxiated guinea pig pups both amniotic fluid and meconium fluid produced only minimal lung parenchymal reaction. However, in the asphyxiated pups there was extensive pulmonary necrosis and diffuse hemorrhage with destruction of alveolar walls. The worse the asphyxia, the greater the degree of pulmonary damage. In the asphyxiated guinea pig pups there was no difference between amniotic fluid and meconium-induced pathologic conditions.[93] As in the severely asphyxiated human fetuses seen in many studies, the type of aspirate, amniotic fluid, or meconium did not acutely affect the severity of the pulmonary disease.[65, 69, 73] The asphyxia was the major element that caused the clinical distress and pathologic damage. Jovanovic and Nguyen[93] concluded "... our results suggest strongly that the extent of lung destruction seen in meconium aspiration syndrome is not related to the aspiration of meconium, but to the length and degree of asphyxia."

Asphyxia may produce pulmonary disease in the newborn through other mechanisms besides pulmonary vascular spasm and vascular hyperreactivity. The reabsorption of fetal lung fluid is critical in the transition from fetal to newborn life. This reabsorption of fluid is dependent on a change in an adenosine triphosphatase–dependent pump in the pulmonary epithelium. This pump may be compromised by asphyxia,[94] and the result would be increased lung fluid and hypoxia. Asphyxia also inhibits capillary flow. Hutchison and Russell[95] demonstrated a 25% reduction in pulmonary capillary blood flow in asphyxiated term infants. As pulmonary capillary blood flow declines, the relative hypoperfusion leads to alveolar damage, which results in injury of type 2 cells, a decrease in surfactant, and atelectasis, producing further hypoxia and the formation of hyaline membranes.[96] The hypoxic damage to the alveolae causes an increase in pulmonary lung permeability and increased lung fluid, which in turn increases hypoxia and leads to right-to-left shunting and the consequent respiratory symptoms.[61, 62, 72, 73, 95]

This question now arises: What is the role of meconium in meconium aspiration syndrome? The presence of meconium in the asphyxiated lung is definitely deleterious, yet in the unasphyxiated lung current evidence suggests that it is rarely harmful. The hypoxia and pulmonary hypoperfusion in the asphyxiated infant impair the normal mechanisms that clear aspirated meconium and amniotic fluid from the lungs. Cilia in the neonatal respiratory tree may be damaged in hypoxia. The meconium that is normally cleared through the pulmonary circulation may remain and induce the chemical pneumonitis that is characteristic of meconium aspiration syndrome.[96] In the alveoli, meconium may act to inhibit and displace surfactant, resulting in atelectasis and hyaline membranes.[72, 97-99] The asphyxiated and injured lung cannot respond well to the insult produced by meconium. Thus asphyxia plus meconium is significantly worse than asphyxia alone. Thibeault et al.[73] studied a group of infants with an initial pH <7.00. Of 14 infants without meconium aspiration, all needed mechanical ventilatory support and four died. Of 25 infants with asphyxia in addition to meconium aspiration, four infants died with persistent fetal circulation. However, among the survivors there was more pulmonary insufficiency, significantly more time on the ventilator, and more respiratory distress than in the infants who were asphyxiated without meconium. Meconium in an injured and severely asphyxiated lung may severely exacerbate the clinical symptoms.

Postdate pregnancies, thick meconium, oligohydramnios, and neonatal disease

The theory that meconium aspiration syndrome is initiated by pulmonary vasoreactivity as a secondary effect of antepartum and neonatal hypoxia helps explain several epidemiologic aspects of meconium aspiration. Among large series of deliveries, respiratory disease rarely develops in infants with meconium but without FHR abnormalities in labor, and the worst disease develops in the infants who are asphyxiated.[11, 15, 19, 64, 100, 101]

The association between postmaturity and meconium passage may be explained by the increased likelihood of cord compression as amniotic fluid declines with advancing gestation, coupled with a more mature fetal intestinal tract.[21, 23, 35, 36, 102-106] Decreasing amniotic fluid volume is a progressive feature of advancing gestation.[8, 16] Druzin and Adams[104] found a 50% incidence of meconium passage in postdate pregnancies with oligohydramnios. Trimmer et al.[105] noted meconium passage in 13% of postdate pregnancies with normal amniotic fluid volume. However, 38% of infants passed meconium in postdate pregnancies with decreased amniotic fluid volume. The decrease in amniotic fluid volume may occur over a matter of 24 hours.[106]

The incidence of meconium aspiration syndrome is increased eightfold in postdate infants in comparison

with term infants, even though the incidence of meconium passage only doubles.[7] The increased incidence of meconium aspiration syndrome with postmaturity is, in part, related to oligohydramnios. Both increased fetal systemic arterial pressure and redistribution of fetal circulation away from the kidneys (and resultant decreased fetal urine output) are a response to hypoxia.[87-90, 92, 104] The decreased urine output results in decreased amniotic fluid and more umbilical cord compression. In one series of pregnancies >42 weeks, 74% of all labors were accompanied by variable decelerations related to cord compressions.[102] Oligohydramnios predisposes to fetal compromise through umbilical cord compression.[102-104] Alternatively, oligohydramnios is in itself a sign of uteroplacental insufficiency.[7, 8, 23, 102, 107, 108]

The consistency of meconium-stained fluid is a product of the amount of meconium passed and the quantity of amniotic fluid in which the meconium is diluted.[6, 8, 13, 16, 21, 23] The less amniotic fluid, the thicker the meconium.[107] In a longitudinal study with amnioscopy, light meconium became thick over time; however, thick meconium did not become thin.[107] Ninety-five percent of meconium aspiration syndrome develops in the presence of thick meconium.[7, 8, 11] Although 5% of meconium aspiration syndrome develops in the presence of thin meconium, the associated respiratory disease is mild.[8, 11]

Meconium aspiration syndrome is particularly severe and often lethal in the presence of infection, because infection may lead to pulmonary hypertension through the direct action of toxins on the pulmonary vasculature.[83, 109] Group B streptococcal exotoxin causes pathologic conditions by the induction of pulmonary vascular spasm. This vascular spasm is mediated through a different pathway than hypoxic vasospasm. Hypoxia and infection may produce compound effects in a logarithmic fashion.[83] Other types of bacteria and viruses have also been associated with pulmonary hypertension.[108, 110, 111] Meconium is also known to inhibit the normal amniotic fluid bacteriostatic qualities, thus promoting bacterial growth.[112] Thus severe or lethal disease may develop in a patient with mild pulmonary insult and meconium inhalation in the presence of infection.[58, 108, 113]

Prevention and treatment

In the period of the late 1950s through the 1960s, when meconium was thought to be a marker of fetal distress, obstetric intervention was called for whenever meconium was noted in the amniotic fluid. In the mid-1970s, authors[114] stated that the presence of meconium was an indication for fetal scalp sampling. Currently, most obstetric protocols call for internal fetal heart monitors in the presence of thick meconium-stained amniotic fluid.[115] Internal monitoring is recommended because of the finding of absent or decreased variability in infants with distress and meconium, patterns more adequately assessed with internal monitors.

In the early 1970s, several investigators thought that meconium aspiration syndrome developed as a result of the infant aspirating meconium with the first breath. Thus protocols were developed calling for nasopharyngeal and oropharyngeal suctioning immediately on delivery of the baby's head, followed by suctioning of the trachea under laryngoscopic visualization by the pediatrician, a "combined approach."[12] If meconium was seen in the trachea, then intubation with subsequent suctioning was advocated. Some authors also advocated routine bronchotracheal saline solution lavage; this is no longer routine.[115]

Although the "combined approach" found widespread acceptance and became the "standard of care," severe disease to develop continued in infants.[10] Many investigators questioned aspects of the combined approach. Hageman et al.[13] found no difference in the clinical outcome of infants with moderate-to-thick meconium who had obstetric suction on the perineum with either the DeLee catheter or a bulb. In 130 infants suctioned by bulb compared with 186 infants suctioned by DeLee catheter, the incidence of meconium below the cords and meconium aspiration syndrome was similar.[13] In addition, respiratory complications developed in three infants from the pediatric suction and tracheal visualization. Locus et al.,[14] in a recent study, also failed to show a superiority between DeLee and bulb suction in removing moderate or thick meconium from the trachea of infants. Falciglia[15] found no difference in the incidence of meconium in the trachea or meconium aspiration syndrome with obstetric suction before or after delivery. Dillard[116] compared the neonatal outcomes of infants not suctioned with those of infants in the series of Gregory et al.,[18] who advocated a combined approach. Dillard found no difference in outcome when infants were not suctioned.

The potential problems of the pediatric aspects of the protocol, DeLee suction and laryngoscopic visualization, were also questioned by a series of reports noting complications. Cordero and Hon[117] studied 46 consecutive neonates who had nasopharyngeal suction. Cardiac arrhythmias developed in seven infants, and five became apneic. Ballard et al.[118] reported a series of eight physicians who were exposed to pathogens from infants' secretions. Organisms ingested by pediatricians included *Neisseria gonorrhoeae,* three cases; herpes simple, one case; varicella, two cases; and *Staphylococcus aureus,* in one. In a survey of pediatricians Ballard et al. found that 74% had ingested secretions during DeLee suction. Van Dyke and Spector[119] reported a case of herpes simplex transmission from pediatrician to

newborn because of suction. Ballard et al.[118] concluded that DeLee suction by mouth was inappropriate and that suction machines should be used.

Further studies did not confirm the original reports of a decrease in the rate of meconium aspiration syndrome or a resolution of the mortality rate after the adoption of the "combined" obstetric-pediatric approach.[1, 4-15, 17, 19] Perinatal advances in the 1970s and 1980s did produce a decrease in the mortality rate of meconium aspiration syndrome. Meconium aspiration syndrome continued to develop in infants in spite of adequate suctioning (Table IV). The explanation as to why the combined approach has been unsuccessful is that meconium is not the primary cause of the pathologic condition of aspiration syndrome; thus removal of meconium does not greatly affect outcome.

With increasing reports of complications from suctioning, as well as questionable efficacy, Linder et al.[17] prospectively studied 572 healthy, unasphyxiated newborns with thick meconium. They compared infants undergoing DeLee suction laryngoscopic visualization and tracheal intubation with infants undergoing DeLee suction only. There were no deaths from meconium aspiration syndrome in either group of infants. In the tracheal visualization group, respiratory complications developed in six infants, meconium aspiration syndrome developed in four, and respiratory complications related to the intubation, stridor, and persistent hoarseness developed in two. In the DeLee-suction-only group, no infant had respiratory complications. The conclusion of the study of Linder et al. was that in healthy newborns laryngoscopic visualization with tracheal suction may be harmful and certainly is not helpful in the prevention of meconium aspiration syndrome. A follow-up review in the *Journal of Pediatrics*[120] concluded that the combined suction protocol should be reevaluated because the etiology of meconium aspiration syndrome may not be associated with the aspiration of meconium, and harm may be done with laryngoscopic visualization.

In a comprehensive study of meconium aspiration syndrome in the armed services from 1973 to 1987, Wiswell et al.[1] showed that there was no change in either the death rate from meconium aspiration syndrome or the incidence of meconium aspiration syndrome for several years after the initiation of a combined obstetric-pediatric protocol in 1973 to 1975. The rate of meconium aspiration syndrome in the period between 1973 and 1976 was lower than at any time until 1985. The death rate in 1975 was also less than the death rate for all years up until 1986. When the years 1973 and 1987 are compared, there was a statistically significant decrease in the incidence of both meconium aspiration syndrome and death from meconium aspiration syndrome. Wiswell et al.[1] stated that although a decrease in the rate of this disease was found, "... these declines were likely influenced by other improvements in perinatal care, which have occurred since the early 1970s. The results do not support that severe meconium aspiration syndrome and resultant deaths can be prevented altogether."

Falciglia[15] stated in 1988 that the rate of meconium aspiration syndrome was similar before and after the incorporation of DeLee and tracheal suctioning. He concluded that meconium aspiration syndrome occurred before birth, and thus the suction was by itself inadequate therapy.

Amnioinfusion is currently being advocated as a treatment for meconium. Sadovsky et al.[121] showed an improvement in arterial cord pH and a reduction in the incidence of meconium below the cords from 29% in 21 fetuses without amnioinfusion to 0% in 9 fetuses with amnioinfusion. Of interest, meconium aspiration syndrome did not occur in either group. In Wenstrom and Parson's study,[122] 36 patients were treated with amnioinfusion and 44 patients were not. Meconium aspiration syndrome developed in three control patients whereas the syndrome did not develop in any infant in the amnioinfusion group. Meconium was present below the cords significantly more frequently in the nonamnioinfusion group. Strong et al.[123] used amnioinfusion in the presence of oligohydramnios and showed a decrease in meconium passage in labor.

Amnioinfusion may help with several of the clinical problems of oligohydramnios. By a decrease in umbilical cord compression there will be less fetal gasping, and by a dilution of the meconium in utero gasping and deep breathing will result in less meconium being inhaled. To assess whether amnioinfusion will decrease the incidence or the severity of meconium aspiration syndrome, large prospective clinical trials will be necessary.[121-123]

Current clinical and experimental data support the theory that meconium aspiration syndrome is a result of intrauterine fetal asphyxia. The best prevention of these conditions is the identification of fetuses who are at risk of asphyxia by means of clinical observation and selective antepartum testing. The postterm pregnancy, as well as other conditions in which oligohydramnios occurs, places patients at particularly high risk for meconium aspiration syndrome. The presence of decreased amniotic fluid in the term and postterm pregnant patient should initiate obstetric intervention. The study of Usher et al.,[7] in which postterm pregnancies were managed with protocols to identify and selectively intervene in cases of oligohydramnios, resulted in no cases of severe meconium aspiration syndrome.

Intrapartum management should include all of the measures that enhance adequate uteroplacental perfusion and reduce the risk of umbilical cord compression. Amnioinfusion in cases of oligohydramnios may be a promising technique for reducing cord compres-

sion, gasping, and intrapartum aspiration of meconium, although additional confirmatory studies are needed. We advocate careful FHR monitoring in the presence of meconium to detect which fetuses may be compromised. Of obvious importance is the timely evaluation of FHR abnormalities that are predictive of fetal asphyxia.

The immediate management of the infant's airway is the most controversial and unsettled matter. It is not clear that the aspiration of meconium before the first breath alleviates in any way the severity of meconium aspiration syndrome; nor is it clear whether any of the methods of removing secretions from the oropharynx and nasopharynx by either obstetrician or pediatrician are effective in preventing or reducing the severity of meconium aspiration syndrome.

Furthermore, there is no convincing evidence that obstetric or immediate neonatal suctioning of the airway to remove meconium improves outcome even in the asphyxiated infant. Clearly, prompt resuscitation and oxygenation are beneficial to such infants. Tracheal intubation and removal of secretions are often necessary to adequately oxygenate the infant. Whether gentle removal of airway secretions, by bulb or DeLee suctioning before delivery of the thorax, reduces neonatal morbidity remains to be shown. Overly vigorous efforts at oropharyngeal or nasopharyngeal suctioning or endotracheal suctioning may be harmful. Local tissue trauma, reflex bradycardia, and delay in providing adequate ventilation are all potential consequences. Well-designed, prospective studies are required to assess the need for and best method of managing the airway of the newly born infant in the presence of meconium-stained amniotic fluid.

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Rapid identification of deoxyribonucleic acid sequence differences in cytochrome P-450 21-hydroxylase (CYP21) genes with denaturing gradient gel blots

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Denaturing gradient gel electrophoresis was used to identify single-base differences in the cytochrome P-450 21-hydroxylase (CYP21) genes of 132 unrelated control individuals and family members of three unrelated patients with 21-hydroxylase deficiency. The salt-wasting variety was caused by gene deletion and gene conversion/deletion mutations in affected members of two families studied. The simple virilizing form, present in the third family, was caused by an apparent point mutation not detectable by routine Southern blots. We have detected many restriction fragment melting polymorphisms in the CYP21 genes of the members of both salt-wasting families and normal individuals with denaturing gradient gel electrophoresis. We also identified a restriction fragment melting polymorphism specific for the simple virilizing patient in the third family. The data demonstrate that the CYP21 genes are highly polymorphic and that denaturing gradient gel electrophoresis is useful for genomic deoxyribonucleic acid analysis of patients with 21-hydroxylase deficiency. (AM J OBSTET GYNECOL 1992;166:184-91.)

Key words: Congenital adrenal hyperplasia, 21-hydroxylase deficiency, denaturing gradient gel electrophoresis

21-Hydroxylase deficiency is the most common cause of congenital adrenal hyperplasia. Classic congenital adrenal hyperplasia is manifested during intrauterine life by varying degrees of aldosterone or cortisol underproduction. Female infants may be adversely affected by masculinization of the external genitalia. Both males and females with the salt-wasting form of 21-hydroxylase deficiency develop sodium depletion and shock after 1 to 2 weeks of life, if left untreated. Prenatal diagnosis and intrauterine treatment of affected female infants will prevent morbidity and mortality associated with this inborn error of metabolism.[1]

Two genes exist for 21-hydroxylation: 21-hydroxylase A (CYP21A) and 21-hydroxylase B (CYP21B).[2, 3] These genes are arranged within the class III region of the histocompatibility complex, in tandem with the two genes coding for the fourth component of complement, C4A and C4B (Fig. 1).[5] Each CYP21 gene consists of 3184 base pairs, differing from each other by 71 bases. Ten of these base pair differences change the protein-coding portion of the sequence.[4, 7] Three deoxyribonucleic acid (DNA) sequence differences in the CYP21A gene disrupt protein translation, making CYP21A a pseudogene. CYP21A and CYP21B have also been called CYP21P and CYP21, respectively, referring to them as the pseudo and active genes.

To date, nearly all of the mutations identified within the 21-hydroxylase genes of patients with congenital adrenal hyperplasia can be attributed to genetic interchange between the CYP21A and CYP21B genes during meiosis.[5, 8-14] Approximately 11% of patients with congenital adrenal hyperplasia have large gene deletions that remove both the CYP21B and C4B genes.[14] Another 11% have large gene conversions in which most of the CYP21B gene sequence has been converted to that of the CYP21A gene.[14] Nearly 76% have point mutations within the CYP21B gene. Many of the point mutations are identical to CYP21A gene mutations.[14]

Only the large gene deletion and conversion mutations are easily identified by routine Southern blotting techniques. These mutations often demonstrate absence of 3.7 kb *Taq* I and 2.9 kb *Kpn* I fragments.[8, 10] Deletions, insertions, and single-base substitutions under 100 base pairs are undetectable on Southern blots,

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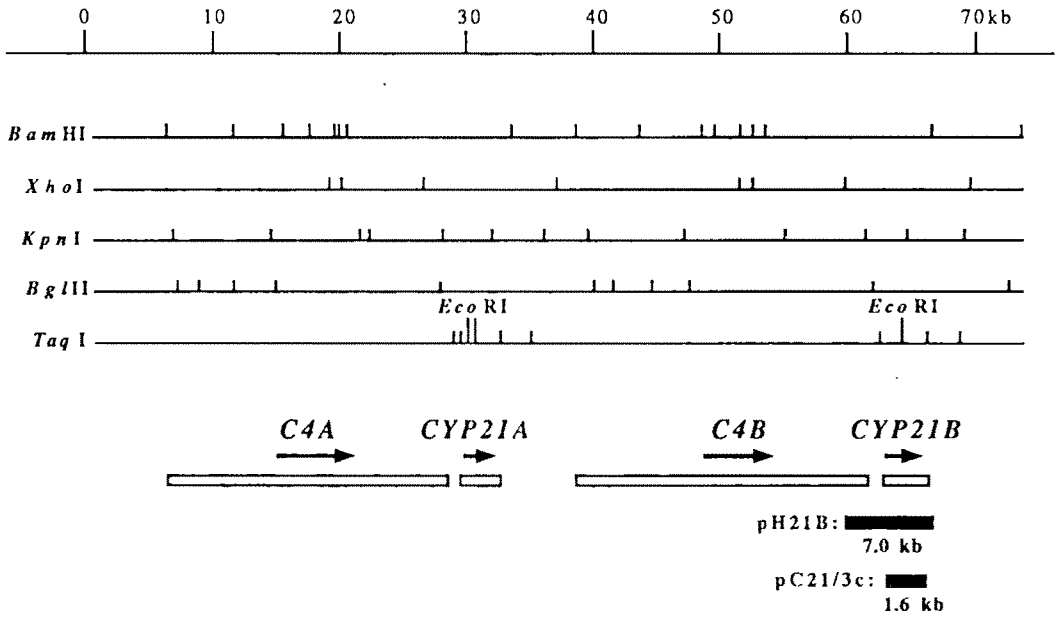


Fig. 1. Structure of CYP21 genes. *Top line* indicates scale in kilobases. Partial restriction map is aligned below scale. Positions of genes at bottom are represented by *open boxes*, with *arrows* indicating direction of transcription. Probes used in experiments are represented by *solid boxes*. This diagram was made with published map and DNA sequence data.[4-6]

unless they change the sequence of certain restriction enzyme recognition sites.[15] The lack of easily identifiable DNA sequence polymorphisms within the 21-hydroxylase genes has limited the use of 21-hydroxylase gene probes for prenatal diagnosis to about 25% of affected chromosomes, by standard Southern blot procedures.[16] Some investigators have suggested that the CYP21 genes are less polymorphic than usual. Consequently, the prenatal diagnosis of congenital adrenal hyperplasia has been made largely by linkage studies that have used DNA probes of closely linked histocompatibility loci.[1, 17]

Denaturing gradient gel electrophoresis can be used to find mutations by detecting small differences in the melting behavior of short (200 to 700 base pairs) DNA fragments.[18] Most DNA fragments subjected to an increasingly denaturing physical environment melt in a discontinuous, stepwise manner, with discrete melting domains of fragments becoming single-stranded in a very narrow range of denaturing conditions. When DNA fragments are electrophoresed in acrylamide gels at high temperatures (60° C) with an increasing gradient of the denaturing solvents formamide and urea, their mobilities sharply decrease as they partially melt, because their shapes become more complex. The denaturant concentration and final gel position, at which a fragment partially melts, are dependent on the DNA sequence of the melted domain. This critical gradient concentration and gel position are often different even when only a single base in the sequence of the melted region is changed. When a mixture of many different fragments is electrophoresed, each will stop at a sequence-dependent position in the denaturing gradient. Fragments containing an A + T–rich domain stop at relatively low denaturant concentrations at a position high on the gel. Conversely, the more stable uniformly G + C–rich fragments usually run to positions low on the gel.

In previous experiments with denaturing gradient gels we physically mapped mutations in the *rosy* locus of *Drosophila melanogaster* to within several hundred base pairs of DNA sequence.[19] The purpose of this study was to apply denaturing gradient gel electrophoresis to identify DNA sequence polymorphisms within the 21-hydroxylase genes of congenital adrenal hyperplasia families and normal control individuals. Because of frequent crossover events between CYP21A and CYP21B genes in normal individuals, it seemed likely that CYP21 gene sequences would be highly polymorphic.[11, 20] Easily detectable polymorphisms would be useful as markers for prenatal diagnosis in the 75% of congenital adrenal hyperplasia chromosomes with point mutations.

Material and methods

This study was approved by the Human Investigational Review Board of New England Medical Center. Informed consent was obtained from all study subjects.

Genomic DNA was extracted from peripheral blood leukocytes with a slightly modified version of a standard method.[6] Samples of 10 ml heparinized blood were mixed with 30 to 35 ml cold cell lysis buffer (0.32

mol/L sucrose, 10 mmol/L Tris hydrochloride [pH 7.5], 5 mmol/L magnesium chloride, and 1% Triton X-100) in 45 ml disposable plastic tubes. Nuclei were pelleted by centrifugation at 2500 revolutions/min for 15 minutes at 4° C. The nuclear pellet was resuspended in 5 ml cold nuclear lysis buffer (75 mmol/L sodium chloride, 24 mmol/L ethylenediaminetetraacetic acid, pH 8.0). Sodium dodecyl sulfate was added to 1% and proteinase K to 100 μg/ml, and the suspension was incubated at 55° C for 1 to 24 hours. The lysate was extracted once in phenol/chloroform (1:1, equilibrated with 0.5 mol/L Tris hydrochloride, pH 8.0), and the DNA was precipitated with isopropanol. The pellet was washed once with 70% ethanol, vacuum dried, and resuspended in TE (10 mmol/L Tris hydrochloride, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.5), at 1 mg/ml. Electrophoresis samples contained 5 to 10 μg of genomic DNA digested in a total volume of 10 to 15 μl. DNA digestions were performed with restriction endonucleases according to the supplier's recommendations.

Digested genomic DNA samples were electrophoresed on 0.8% agarose gels. After pretreatment of the gels, denatured restriction fragments were transferred onto Nytran (Schleicher and Schuell, Keene, N.H.) by Southern blotting.[21] Membranes were vacuum dried at 80° C for 1 to 2 hours.

Denaturing gradient gels were prepared as previously described.[19] The apparatus used for the preparation and electrophoresis of the denaturing gradient gels was designed by Leonard Lerman and purchased from Green Mountain Lab Supply (Waltham, Mass.).[22] All of the denaturing gradient gels were 6.5% acrylamide (37.5:1 acrylamide/bisacrylamide) in TAE buffer (40 mmol/L Tris acetate, 20 mmol/L sodium acetate, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.4). The range of denaturant concentration in the gels was at least 20% to 80% (100% denaturant concentration is 7 mol/L urea and 40% formamide). DNA samples were electrophoresed at 75 to 80 V for 16 to 18 hours at 60° C. Afterward, the gels were stained in ethidium bromide (1 μg/ml) for 5 minutes and examined over a long-wave ultraviolet light source.

The destained denaturing gradient gels were soaked in 0.5 mol/L sodium hydroxide for 5 minutes, 0.5 mol/L Tris, pH 8.0, for 5 minutes, and then in transfer buffer (20 mmol/L Tris, 1 mmol/L ethylenediaminetetraacetic acid, pH 8.0) for at least 5 minutes. Next, each gel was placed in a stack with a nylon hybridization membrane (Nytan), and the stack was inserted into an electrophoretic Western blot transfer apparatus. DNA fragments were transferred by electrophoresis at 600 mA for 2 hours. After electrotransfer, the DNA blots were rinsed briefly in 6× saline sodium citrate buffer (0.15 mol/L sodium chloride, 15 mmol/L sodium citrate, pH 7.4) and baked under a vacuum at 80° C for 1 to 2 hours.

Plasmids containing CYP21B gene inserts were kindly supplied to us by Dr. Perrin White. The plasmid pH21B includes a 7.0 kb *Xho* I/*Bam*HI fragment that contains the entire CYP21B gene and part of the C4B gene as well (Fig. 1).[7] The plasmid pC21/3c contains a 1.6 kb *Bam*HI fragment that includes the CYP21B complementary DNA (Fig. 1).[7] Both insert fragments were excised from their respective plasmids and purified by acrylamide gel electrophoresis. Purified DNA fragments were labeled with phosphorus 32 with the method of primer oligonucleotide extension after hybridization with random primers.[23]

The blots were hybridized as previously described.[19, 24] After hybridization, the blots were washed as follows: (1) 2× saline sodium citrate plus 1% sodium dodecyl sulfate at 22° C for 30 minutes, (2) 1× saline sodium citrate plus 1% sodium dodecyl sulfate at 68° C for 30 minutes, and (3) 0.1× saline sodium citrate plus 1% sodium dodecyl sulfate at 68° C for 30 minutes. Blots were exposed to x-ray film at −70° C in the presence of an intensifying screen for 1 to 10 days.

Results

Study subjects. Control DNA was prepared from peripheral blood leukocytes of 132 unrelated male and female volunteers, not known to have any form of congenital adrenal hyperplasia. A second group of study subjects consisted of members of three families with at least one individual diagnosed with 21-hydroxylase deficiency: families K (n = 3, two generations), T (n = 10, four generations), and M (n = 4, two generations). These three families were selected because they were thought to have at least one of the common types of 21-hydroxylase gene mutations associated with classic congenital adrenal hyperplasia. Salt-wasting 21-hydroxylase deficiency was manifested in affected members of the T and K families and was caused by gene deletion and gene conversion-deletion mutations, respectively. The simple virilizing congenital adrenal hyperplasia manifested by the M family proband was caused by an apparent point mutation.

Southern blot analysis. Genomic DNA from normal and subjects with congenital adrenal hyperplasia was examined for large deletions and chromosomal rearrangements in the 21-hydroxylase genes with Southern blots. Southern blot results were compared with the congenital adrenal hyperplasia classification of Morel et al.[14]

Control subjects. Genomic DNA from all normal individuals tested had the expected CYP21A and CYP21B gene-specific 3.2 and 3.7 kb *Taq* I fragments, respectively, and the CYP21A and CYP21B gene-specific 4.0 and 2.9 kb *Kpn* I fragments, respectively (data

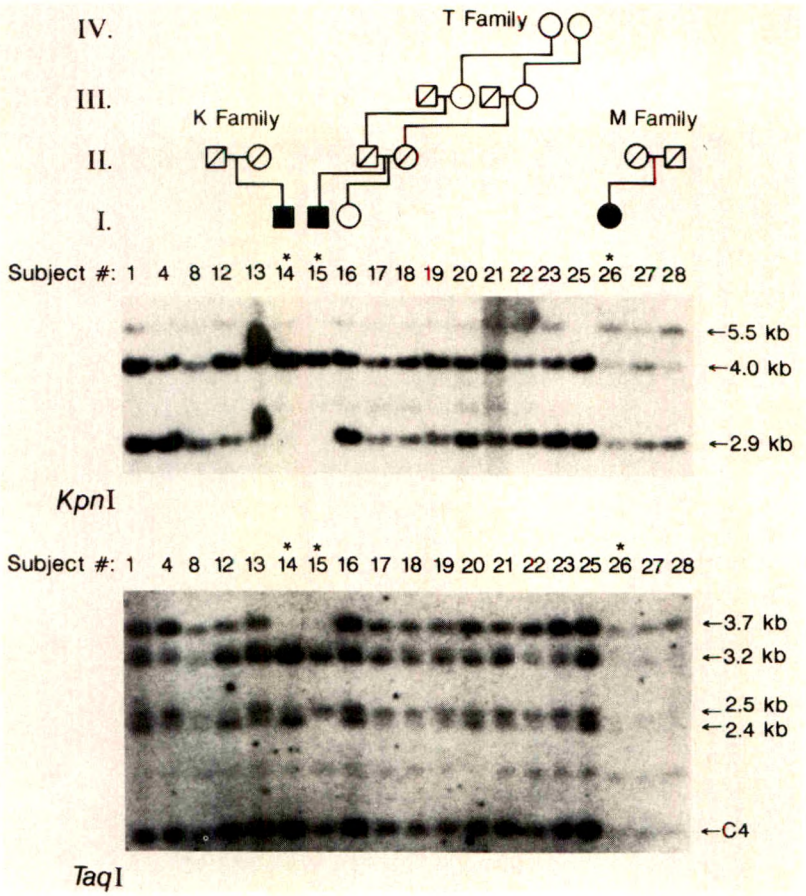


Fig. 2. *Kpn* I and *Taq* I restriction analysis of congenital adrenal hyperplasia pedigrees and controls. *Controls,* Subjects 1, 4, and 8 are control individuals demonstrating the CYP21A 4.0 kb *Kpn* I fragment and 3.2 and 2.5 kb *Taq* I fragments. CYP21B 2.9 kb *Kpn* I fragment and 3.7 and 2.4 kb *Taq* I fragments are also present. *K family,* Affected son is a compound heterozygote for gene conversion and gene deletion mutations. Mutant paternal CYP21 haplotype contains two CYP21A alleles: two copies of 4.0 kb *Kpn* I fragment and 3.2 and 2.4 kb *Taq* I fragments. Mutant maternal CYP21 haplotype is CYP21B gene deletion, with absence of the 2.9 kb *Kpn* I and 3.7 and 2.5 kb *Taq* I fragments. *T family,* Affected son is homozygous for CYP21B gene deletion. All CYP21B fragments are deleted. *M family,* Affected daughter has point mutations not detectable by *Kpn* I and *Taq* I Southern blot analysis.

not shown). The expected downstream CYP21A and CYP21B gene-specific 2.4 and 2.5 kb *Taq* I fragments, as well as the C4 fragment, were present in all normal individuals. Hybridization intensities for the CYP21A and CYP21B gene-specific fragments were approximately equal in most (e.g., Fig. 2, subject 1), but not all (e.g., subject 4), normal individuals. This suggests that unequal copy numbers of the two 21-hydroxylase genes are common among normal individuals. Additionally, because control subjects did not undergo adrenocorticotropin stimulation testing, it is possible that several of these individuals were heterozygote carriers of CYP21 gene mutations.

K family: Gene conversion-deletion. The affected son lacked a normal CYP21B gene (Fig. 2, subject 14). Whereas a densitometric comparison of CYP21A and CYP21B gene fragments was not performed, a visual comparison of band intensities suggests that the affected son's paternal sixth chromosome had two copies of the CYP21A gene and was missing CYP21B. This was demonstrated by three doses each of the 4.0 kb *Kpn* I fragment and the 3.2 and 2.4 kb *Taq* I fragments and only one dose each of the 2.9 kb *Kpn* I fragment and the 3.7 and 2.5 kb *Taq* I fragments (Fig. 2, subject 12). This hybridization pattern is likely a variant of Morel's gene conversion, congenital adrenal hyperplasia haplotype IV.[14] The affected subject's maternal sixth chromosome had a large deletion of DNA originating in the 2.4 kb *Taq* I fragment downstream from the CYP21A gene, and ending within the 3.7 kb *Taq* I fragment of CYP21B gene (Fig. 2, subject 13). This phenotype is consistent with Morel's congenital adrenal hyperplasia haplotype V, a deletion of approximately 30 kb extending from the middle of the CYP21A pseu-

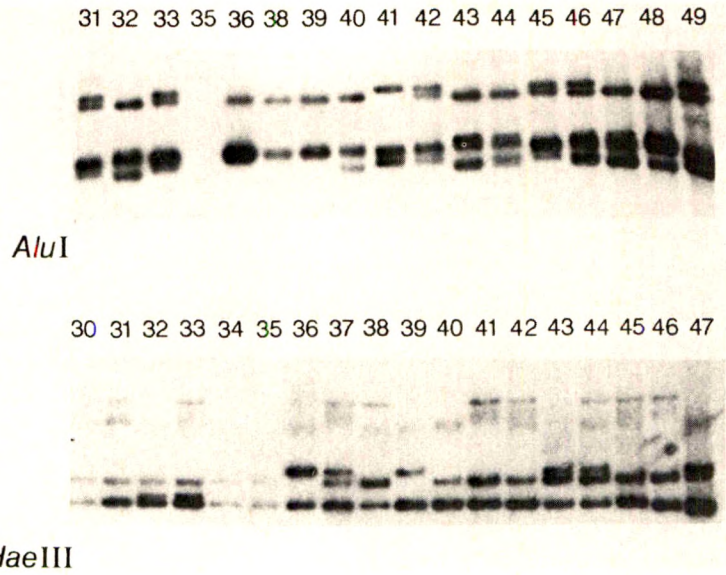


Fig. 3. Denaturing gradient gel electrophoresis blot of *Alu* I and *Hae* III digests of control subjects' genomic DNA. *Alu* I patterns demonstrate melting polymorphism for both upper and lower cluster of fragments. Five distinct fragment melting patterns are represented. *Hae* III patterns demonstrate melting polymorphism, with at least seven different melting patterns present.

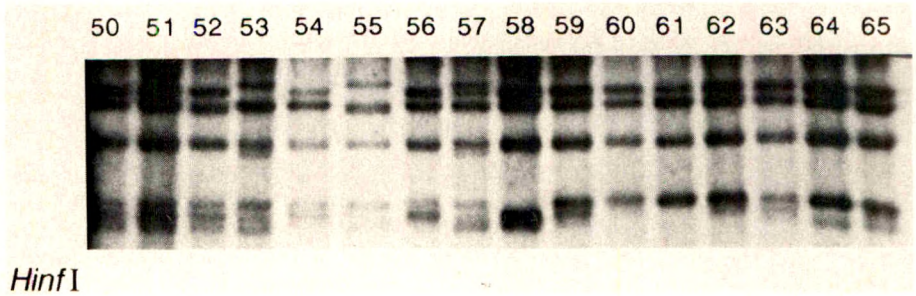


Fig. 4. Denaturing gradient gel electrophoresis blot of *Hin*fI digest of control subjects' genomic DNA. Extensive melting polymorphism is present, with at least seven different *Hin*fI melting patterns represented.

dogene to a corresponding position in the middle of the CYP21B gene. Both of the affected individual's sixth chromosomes were probably inherited intact from the parents, without any recombination or further gene conversion in the CYP21 region (Fig. 2, subject 14).

T family: Homozygous deletion. The affected son was homozygous for a deletion that removes most or all of the CYP21B gene, extending through the C4B gene to the 3′-end of the CYP21A gene. The affected son lacked both the 3.7 and 2.4 kb *Taq* I fragments and also the 2.9 kb *Kpn* I fragment (Fig. 2, subject 15). Genomic DNA from both parents (Fig. 2, subjects 17 and 18), the paternal grandfather (subject 19), and the maternal grandfather (subject 21) demonstrated decreased hybridization intensities for the three fragments absent in the affected son. This deletion corresponds to Morel's congenital adrenal hyperplasia haplotype V as discussed above.[14]

M family: Point mutation or small gene conversion. The affected daughter, unaffected sister (data not shown), and both parents (Fig. 2, subjects 26, 27, and 28) demonstrated normal CYP21 gene *Taq* I and *Kpn* I fragments. This result matches Morel's point mutation congenital adrenal hyperplasia haplotype I.[14] Genomic DNA from the M family was digested with several additional restriction enzymes (*Bgl*I, *Hin*dIII, *Bam*HI, and *Eco*RI), and analyzed on Southern blots. These blots were not different from the blots of normal individuals (data not shown).

Denaturing gradient gel blot analysis

Control subjects. Genomic DNA samples from normal, unrelated volunteers, digested with the restriction enzymes *Alu* I ($n = 84$), *Hae* III ($n = 55$), *Hin*fI ($n = 45$), and *Rsa* I ($N = 40$) were electrophoresed on denaturing gradient gels. Blots prepared from these gels were hybridized with a radioactive probe prepared from CYP21 gene complementary DNA.

In striking contrast to our Southern blot data, most small CYP21 gene fragments from normal, unrelated individuals were polymorphic on denaturing gradient



Fig. 5. Denaturing gradient gel electrophoresis blot of *Alu* I and *Hae* III digests of genomic DNA for congenital adrenal hyperplasia pedigrees and controls. *Controls,* Subjects 10 and 11 are control individuals and demonstrate different fragment melting patterns for each digest. Melting pattern differences are seen in K and T family members and can be followed through each generation represented. *K family (left pedigree),* Affected son is compound heterozygote for gene conversion and gene deletion mutation. Only upper *Alu* I fragment is missing. *T family (middle pedigree),* Affected son is homozygous for CYP21B gene deletion. Both upper fragments in *Alu* I and *Hae* III digests are missing. *M family (right pedigree),* Affected daughter has point mutations. *Alu* I and *Hae* III fragments of her genomic DNA do not differ from those of her parents.

gel blots. Each lane of the blot in Fig. 3 showed polymorphic (two or more gel position phenotypes) *Alu* I fragments. The top fragment in each lane had distinct alleles, with the lower form more common in this group of individuals. The lower cluster of *Alu* I bands was caused by either multiple alleles of a single fragment or a group of several different fragments, with at least one having polymorphic alleles. Taken together, the *Alu* I fragments revealed five distinctive fragment melting patterns. *Hae* III fragments were similarly polymorphic in control individuals (Fig. 3). At least seven different melting patterns were present in the control individuals shown. *Hin*fI digestion of genomic DNA from normal control subjects also revealed extensive polymorphism, as demonstrated by at least seven different melting patterns (Fig. 4). Among three polymorphic *Rsa* I fragments, at least seven different melting patterns were found in normal control DNA (data not shown).

Congenital adrenal hyperplasia family members. Denaturing gradient gel blots of genomic DNA of the K, T, and M congenital adrenal hyperplasia family members were prepared and hybridized with the CYP21 gene complementary DNA probe. *Alu* I and *Hae* III digests electrophoresed on denaturing gradient gels demonstrated an array of melting polymorphisms similar to that found in normal individuals (Fig. 5). The highest *Alu* I fragment was missing in the affected T and K males (Fig. 5, subjects 14 and 15). This result suggests that the missing fragment originated from the CYP21B gene. The presence of this *Alu* I fragment, in either of the two allelic forms, was associated with the absence of congenital adrenal hyperplasia in the T and K families, through four generations.

No restriction fragment melting polymorphisms were found in the *Alu* I and *Hae* III digests of genomic DNA from M family members. The M family was studied further with the restriction enzymes *Hin*fI, *Rsa* I, *Bst*NI, *Sau*3A, *Pst* I, and *Sau*96I. Except for the *Bst*NI digests, the patterns were identical in each set of digests of DNA from the original three M family members, with the addition of an unaffected daughter (Fig. 6). The presence of the lowest *Bst*NI band was associated with congenital adrenal hyperplasia in this small family.

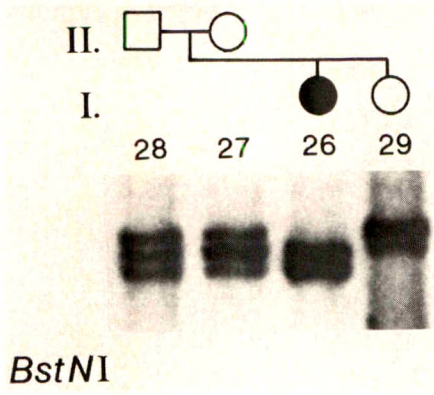


Fig. 6. Denaturing gradient gel electrophoresis blot of *Bst*NI digests of M family genomic DNA. Melting differences are noted between M family members after denaturing gradient gel electrophoresis in *Bst*NI digests only. Restriction fragment melting polymorphism shown here marks CYP21B allele that segregates with congenital adrenal hyperplasia in this family. DNA from the unaffected daughter is included here.

This restriction fragment melting polymorphism marked the CYP21B allele that segregated with congenital adrenal hyperplasia.

Comment

Prior studies that used routine Southern blot analysis demonstrated relatively few easily identifiable polymorphisms within the CYP21 genes. The data presented here with molecular analysis of denaturing gradient gel electrophoresis demonstrate that numerous DNA sequence polymorphisms exist within the CYP21 genes of normal and patients with congenital adrenal hyperplasia. By presenting one family with each of the major types of 21-hydroxylase deficiency mutations, we have demonstrated the mendelian inheritance of these polymorphisms. Furthermore, we have demonstrated the potential clinical usefulness of denaturing gradient gel electrophoresis for the molecular analysis of the CYP21 genes in families affected with 21-hydroxylase deficiency. The rapid identification of single-base differences in the 21-hydroxylase genes will permit a CYP21 gene-specific prenatal diagnosis.

The denaturing gradient gel electrophoresis analysis of the CYP21 genes demonstrated polymorphisms for more than half of the fragments produced by digestion with each of the restriction enzymes tested. These genes appeared to be more highly polymorphic than other autosomal genes studied (Gray MR. Unpublished data). The polymorphisms found within the DNA of control individuals were also present in families carrying each of the three major types of CYP21 gene mutations. Although the T and K families carry CYP21B gene deletion and large gene conversion mutations, our results for the affected T and K individuals suggest that many of the CYP21 gene restriction fragment melting polymorphisms were in the CYP21A pseudogene. CYP21B-specific fragments were also identified: The T male with a gene deletion lacked the highest bands shown in the *Hae* III and *Alu* I blots. The K male retained the highest *Hae* III fragment but lost the highest *Alu* I fragment.

The T and K family denaturing gradient gel electrophoresis results revealed fragments specific for deletion and conversion mutations. Denaturing gradient gel electrophoresis of *Alu* I and *Hae* III digests distinguished between deletion and conversion mutations. Deletion mutations may result in absence of both highest *Alu* I and *Hae* III fragments, and large conversion mutations may result in absence of the *Alu* I fragment only. Further denaturing gradient gel electrophoresis studies of other CYP21 gene deletion and conversion chromosomes are needed to test this finding.

The data on the M family demonstrated that denaturing gradient gel electrophoresis analysis of the 21-hydroxylase genes can be used to identify the affected CYP21 point mutation allele for prenatal diagnosis. Although the *Bst*NI restriction fragment melting polymorphism in the affected female was not likely to be the specific mutation causing congenital adrenal hyperplasia, it was an intragenic marker for the mutation in this family. Denaturing gradient gel electrophoresis can also provide a rapid means of identifying the mutant region of CYP21B and permit a more rapid sequence determination of this specific region.

Recent studies with CYP21A-specific oligonucleotide probes and densitometric analysis of CYP21A and CYP21B-specific gene fragments have provided evidence of frequent genetic interchange between the two 21-hydroxylase genes, even in normal individuals.[11, 20] The extensive number of polymorphisms found by denaturing gradient gel electrophoresis is consistent with observations of extensive genetic activity in the region of the CYP21 genes.

Use of denaturing gradient gel electrophoresis will greatly augment our ability to identify 21-hydroxylase gene mutations. This is important for the 76% of congenital adrenal hyperplasia chromosomes with point mutations not detectable on routine Southern blots.[14] Histocompatibility linkage studies, presently required for prenatal diagnosis of such point mutations, will be enhanced by the adjunctive use of denaturing gradient gel electrophoresis and potentially replaced by this technique. The more direct denaturing gradient gel electrophoresis method permits intragenic diagnosis of congenital adrenal hyperplasia and eliminates the potential misdiagnosis caused by recombination between histocompatibility loci and CYP21B.[5]

In summary, our data provide evidence that the DNA sequences of the 21-hydroxylase genes are highly polymorphic in all individuals. Our data demonstrate the

potential clinical usefulness of denaturing gradient gel electrophoresis for the intragenic prenatal diagnosis of 21-hydroxylase deficiency.

We thank Dr. Perrin White for providing the CYP21 gene probes.

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Transforming growth factor-α and epidermal growth factor messenger ribonucleic acid and protein levels in human placentas from early, mid, and late gestation

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OBJECTIVE: Human placenta expresses receptors for transforming growth factor-α and epidermal growth factor throughout pregnancy. Experiments were performed to determine whether epidermal growth factor or transforming growth factor-α might be synthesized by placental cells and act through an autocrine mechanism to influence functioning of placental cells in vivo.

STUDY DESIGN: Human placentas from early, mid, and late gestations were analyzed for transforming growth factor-α and epidermal growth factor messenger ribonucleic acid and proteins. Polyadenylic acid–positive ribonucleic acid was isolated from placentas from 10, 11, 13, 21, 32, 38, 39, and 40 weeks of gestation and analyzed by Northern analysis for hybridization with complementary deoxyribonucleic acid probes specific for epidermal growth factor or transforming growth factor-α. Levels of immunoreactive epidermal growth factor and transforming growth factor-α were measured by specific radioimmunoassays in pools of placentas from early, mid, and late gestations, and levels of epidermal growth factor and transforming growth factor-α receptor–active protein were measured by radioreceptor assay.

RESULTS: All placentas had a strong transforming growth factor-α hybridization band at 4.5 kb and a weak epidermal growth factor hybridization band at 5.2 kb. High levels of transforming growth factor-α immunoreactive protein (90 to 180 ng/mg protein) and low levels of immunoreactive epidermal growth factor (3 to 9 pg/mg protein) were detected in pools of placentas from early, mid, and late gestations. High levels of epidermal growth factor and transforming growth factor-α receptor–active protein (250 ng/mg protein) were also detected.

CONCLUSION: Human placentas contain relatively high levels of immunoreactive and receptor–active transforming growth factor-α, as well as transforming growth factor-α messenger ribonucleic acid, throughout gestation. This finding suggests that transforming growth factor-α may act by an autocrine system to influence human placental cell function in vivo. (Am J Obstet Gynecol 1992;166:192-9.)

Key words: Growth factors, transforming growth factor-α, epidermal growth factor, placenta, messenger ribonucleic acid, autocrine systems

Transforming growth factor-α (TGF-α) and epidermal growth factor (EGF) are structurally related polypeptides that bind to a common cellular receptor protein, the EGF–TGF-α receptor.[1, 2] They are potent mitogens for many types of cells, including epithelial cells and fibroblasts. In addition to their mitogenic action, EGF and TGF-α can influence specialized functions of several types of differentiated cells. EGF rapidly stimulated prolactin synthesis and inhibited growth hormone synthesis in rat pituitary cell cultures[3] and inhibited gastric acid secretion by guinea pig parietal cells.[4]

Several lines of evidence suggest that TGF-α or EGF may play important physiologic roles in fetal-placental development and function. Human placental membranes express high levels of EGF–TGF-α receptors throughout gestation.[5-7] Although the physiologic actions of EGF or TGF-α on human placental cells are not completely understood, they may influence synthesis of placental hormones. EGF stimulated secretion of chorionic gonadotropin and prolactin by normal hu-

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man placental trophoblasts in short-term culture.[8-10] In addition, EGF stimulated synthesis of progesterone by JEG-3 human choriocarcinoma cells in culture[11] and attenuated follicle-stimulating hormone–induced estrogen synthesis by rat granulosa cells.[12] The effects of TGF-α on placental cell functions have not been examined.

The source(s) of TGF-α or EGF that could influence human placental function in vivo has not been determined. EGF or TGF-α could be produced by maternal cells and delivered to the placenta through the maternal circulation by a typical endocrine mechanism. The growth factors also could be synthesized by fetal syncytiotrophoblast cells or produced by maternal decidua cells and act by autocrine or paracrine mechanisms to influence fetal syncytiotrophoblasts.

Initial reports of blood levels of EGF indicated that plasma levels of immunoreactive EGF material in nonpregnant women were very low (30 pmol/L) and did not increase during pregnancy.[13] Subsequent studies demonstrated that immunoreactive EGF material detected in platelet-rich plasma and serum was generated from degranulated platelets, which suggested that plasma does not normally contain physiologically significant levels of EGF.[14, 15] Plasma levels of immunoreactive TGF-α from normal individuals were recently reported to be approximately 5 pmol/L, which is below the level of TGF-α that significantly stimulates mitosis of most cells in vitro.[16] Plasma levels of TGF-α have not been reported for pregnant women. Thus it seems unlikely that placental cells would be influenced significantly by either EGF or TGF-α in maternal blood.

Results of experiments in mice and rats suggest that TGF-α or EGF may influence rodent placental cells by autocrine or paracrine mechanisms. Before development of the placenta, TGF-α messenger ribonucleic acid (mRNA) and protein (but not EGF, basic fibroblast growth factor, nerve growth factor, granulocyte colony-stimulating factor) were detected in mouse preimplantation blastocysts.[17] After implantation of rat embryos, high levels of TGF-α mRNA were detected in rat maternal decidua; then mRNA levels slowly declined as the decidua was resorbed.[18] At day 14 of gestation, low levels of TGF-α mRNA were also detected in rat placental tissue. Human placenta has not been analyzed for TGF-α and EGF mRNA and proteins.

To help assess if EGF or TGF-α might interact with human placental cells by autocrine or paracrine mechanisms, we used complementary deoxyribonucleic acid (cDNA) hybridization techniques and specific radioimmunoassays (RIA) and radioreceptor assays to analyze samples of normal human placental tissues obtained during early, mid, and late gestation for the presence of TGF-α and EGF mRNA and proteins.

Material and methods

Placental tissue collection. Early human placental material was obtained from curettage of the uterus during the first trimester of pregnancy. Placentas from mid and late gestation were obtained at delivery. Care was taken to select only fetal placental tissue. Placental tissues were immediately washed with saline solution, frozen in liquid nitrogen, and then stored at −70° C. The gestational age was determined from the date of the last menstrual period or from clinical or ultrasonographic evaluation. Tissues were procured under appropriate guidelines of the Human Study Committee and the Animal Care and Use Committee of the University of Louisville and the University of Florida.

Preparation of ribonucleic acid (RNA). Total RNA was prepared from frozen tissues by slight modifications of the method of Rall et al.[19] Briefly, tissues were homogenized in 10 volumes of 5 mol/L guanidinium thiocyanate containing 100 mmol/L Tris hydrochloride, pH 7.6, 10 mmol/L ethylenediaminetetraacetic acid (EDTA), and 14% 2-mercaptoethanol. Homogenates were centrifuged at 10,000*g* for 10 minutes at 4° C and RNA precipitated from the supernatants by overnight incubation at 4° C in 5.5 volumes of 4 mol/L lithium chloride. Pellets were resuspended and washed with a solution of 3 mol/L lithium chloride containing 4 mol/L urea until the supernatant was clear. Samples were dissolved in 10 mmol/L Tris hydrochloride, pH 8.0, and 0.5% sodium dodecyl sulfate and then digested with protease K (200 μg/ml) for 30 minutes at 30° C. RNA was further deproteinized by extraction with phenol/chloroform/isoamyl alcohol (1:1:0.1) and recovered by overnight precipitation with 66% ethanol and 33% 0.1 mol/L sodium chloride. RNA samples were stored at −20° C in ethanol–sodium chloride.

Polyadenylic acid–positive RNA was isolated from total RNA by oligo(dT)-cellulose chromatography.[20] Samples of total RNA were dissolved in 500 mmol/L sodium chloride, 10 mmol/L Tris hydrochloride, pH 7.6, 1 mmol/L EDTA, and 0.5% sodium dodecyl sulfate (binding buffer) and mixed for 30 minutes with the oligo(dT)-cellulose resin (previously equilibrated with binding buffer). Suspensions of resin were then poured into columns and washed successively with binding buffer, intermediate buffer (100 mmol/L sodium chloride, 10 mmol/L Tris hydrochloride, pH 7.6, 1 mmol/L EDTA, 0.5% sodium dodecyl sulfate), and elution buffer (10 mmol/L Tris hydrochloride, pH 7.6, 1 mmol/L EDTA, 0.5% sodium dodecyl sulfate. Polyadenylic acid–positive RNA was recovered by precipitation of the elution buffer with ethanol–sodium chloride. RNA concentrations were determined by absorbance at 260 nm (one A_{260} unit equaling 40 μg/ml RNA).

Hybridization analysis. Northern blots were performed as described with slight modifications.[20] Briefly, samples of polyadenylic acid–positive RNA (5 μg) were denatured by heating at 55° C for 15 minutes in a solution containing 50% formamide, 2.2 mol/L formaldehyde, 20 mmol/L MOPS buffer (3-{N-morpholino}propanesulfonic acid), pH 7.0, 5 mmol/L sodium acetate, and 0.5 mmol/L EDTA. Samples were subjected to electrophoresis at 35 V for 20 hours through a 1% agarose gel containing 2.2 mol/L formaldehyde, 40 mmol/L MOPS, pH 7.0, 10 mmol/L sodium acetate, and 1 mmol/L EDTA. Total placental RNA was also subjected to electrophoresis and analyzed by ethidium bromide staining to determine location of 18S and 28S ribosomal size markers. RNA was blotted onto nylon filters by capillary transfer and fixed by heating at 80° C. Blots were prehybridized at 42° C for 2 hours in a solution containing 50% formamide, 5× SSPE (1× SSPE is 0.18 mol/L sodium chloride, 10 mmol/L sodium phosphate, pH 7.7, and 1 mmol/L EDTA), 5× Denhardt's solution (50× Denhardt's is 1% acetylated bovine serum albumin, 1% ficoll, and 1% polyvinylpyrrolidone), 0.1% sodium dodecyl sulfate, and denatured salmon sperm deoxyribonucleic acid (DNA) (200 μg/ml). Hybridization was performed for 16 hours at 42° C in the same solution but containing 10% dextran sulfate and 10^6 counts/min/ml of phosphorus 32–labeled TGF-α or EGF probe. After hybridization, blots were washed four times with 2× SSPE containing 0.1% sodium dodecyl sulfate for 5 minutes each and twice with 0.2× SSPE and 0.1% sodium dodecyl sulfate at 65° C for 15 minutes each. Autoradiograms were generated by exposing the blots to Kodak AR (Eastman Kodak, Rochester, N.Y.) film with DuPont (DuPont Instruments, Wilmington, Del.) intensifying screen for 24 hours at −80° C.

TGF-α and EGF cDNA probes. The rat TGF-α cDNA probe was kindly provided by Oncogen (Seattle) and consisted of a 2.3 kb *Eco*RI insert subcloned into pEBBL.[21] The TGF-α cDNA clone encodes the entire 159 amino acid precursor TGF-α molecule and has been shown to hybridize to a single species of approximately 4.5 kb with rat RNA. The gene for human TGF-α also has been cloned and was found to encode for a 160 amino acid precursor polypeptide.[22] Hybridization of a human TGF-α cDNA probe to polyadenylic acid–positive RNA obtained from normal human skin also generated a single band of approximately 4.5 to 4.8 kb.[23]

The human EGF cDNA probe was kindly supplied by Chiron Corporation (Emeryville, Calif.). It encodes the entire 1207 amino acid precursor form of EGF and consisted of a 6.4 kb *Sma* I insert cloned into pSAD.[24] The probe has been shown to hybridize to a single transcript of 5.0 to 5.2 kb from adult human kidney polyadenylic acid–positive RNA.[24] Both the TGF-α and EGF cDNA probes were radiolabeled to a specific activity of approximately 10^9 counts/min/μg with an oligonucleotide random priming kit (Amersham Corp., Arlington Heights, Ill.).

RIAs for EGF and TGF-α. Individual placentas from fetuses at early and mid gestation were too small to permit RIAs for both EGF and TGF-α. Thus samples of placental tissue were formed by combining individual placentas of early, mid, and late gestation. Each pooled sample contained 5 gm of tissue. The sample from early gestation contained tissue from nine placentas of 9 to 13 weeks' gestation. The samples from mid and late gestation each contained tissue from six placentas of 21 to 32 weeks' gestation and 33 to 40 weeks' gestation, respectively.

TGF-α immunoreactive protein was measured with an RIA kit specific for TGF-α, obtained from BioTope (Seattle). Placental samples were extracted with denaturing detergent and reduced according to manufacturer's instructions, and samples were assayed in triplicate at three dilutions. Displacement data were linearized by logit transformation, and the best fit line was determined by linear regression analysis. Slopes of the lines generated by placental samples and by the TGF-α standard were compared for differences by *t* test for slopes.[25] Samples that produced displacement lines that were parallel to TGF-α standard were considered to contain immunoreactive TGF-α. Levels of immunoreactive TGF-α protein were expressed as nanograms of immunoreactive TGF-α per milligram of protein determined by BCA protein assay (Pierce Chemical Co., Rockford, Ill.). The half-maximal displacement occurred at approximately 20 nmol/L TGF-α, and nonspecific binding was approximately 3% of total binding. All samples were analyzed in a single assay, and the intraassay coefficient of variation was 3%. The TGF-α RIA used polyclonal goat antiserum generated to the C-terminal 17 amino acid sequence of TGF-α (amino acids 34 to 50). The RIA is highly specific for TGF-α (no cross-reactivity with mouse or human EGF at 1 μmol/L).

EGF immunoreactive protein was measured with minor modifications to a solid-phase RIA described previous.[26] Briefly, placental samples were extracted with 1% Triton X-100 detergent in phosphate-buffered saline (PBS) solution and assayed in triplicate at three dilutions. Displacement data were linearized by logit transformation, and slopes of lines determined by linear regression analysis were analyzed for differences with *t* test for slopes as described. Levels of immunoreactive EGF protein were expressed as nanograms of immunoreactive EGF per milligram of protein determined by BCA protein assay. Half-maximal displacement for the assay was approximately 300 pmol/L, and nonspecific binding was <15%. All placental samples

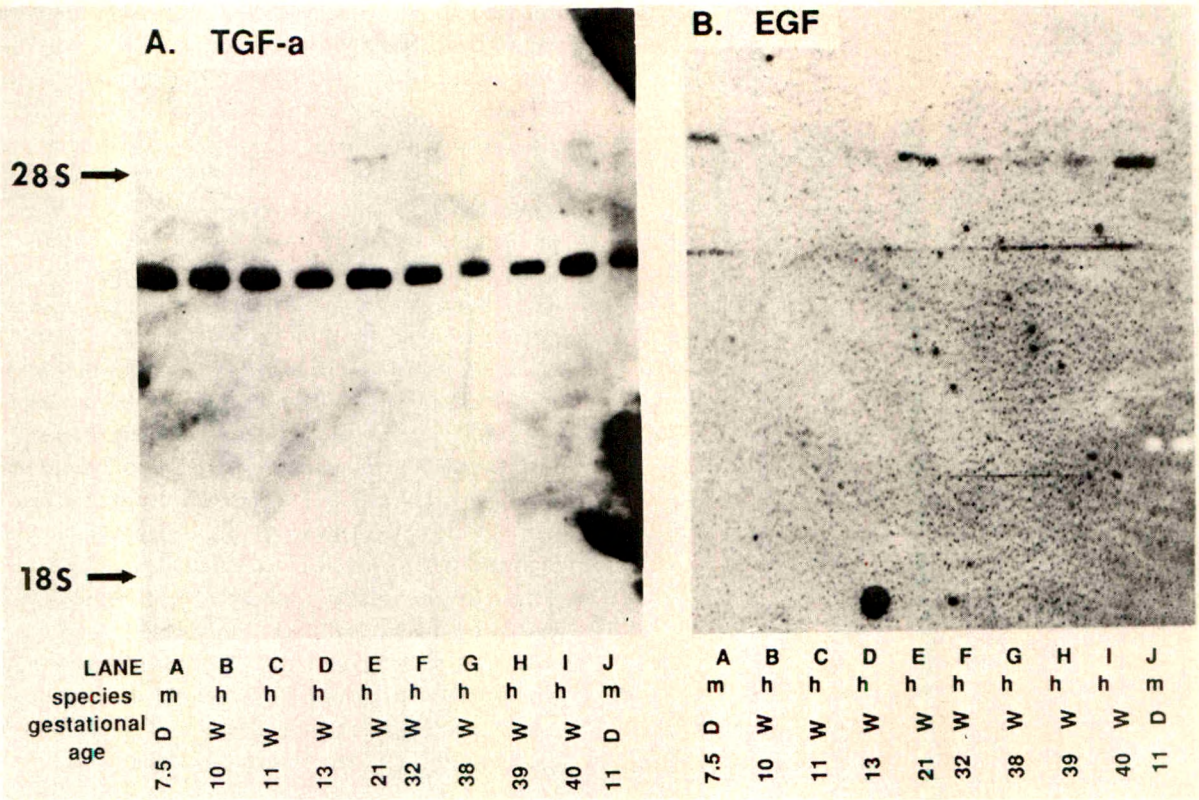


Fig. 1. Northern analysis of placental polyadenylic acid–positive RNA for TGF-α and EGF mRNA. Polyadenylic acid–RNA (5 μg per lane) was isolated from mouse *(lanes A and J)* or human *(lanes B through I)* placentas of varying gestational ages (*lane A*, 7.5 days; *lane J*, 11 days; *lanes B through I*, 10, 11, 13, 21, 32, 38, 39, and 40 weeks, respectively). Blots were hybridized under high-stringency conditions with cloned cDNA for rat TGF-α **(A)** and human EGF **(B).** TGF-α probe in **A** hybridized to 4.5 kb mRNA and EGF probe hybridized to 5.2 kb mRNA.

were analyzed in a single assay, and the intraassay coefficient of variation was approximately 10%. The EGF RIA used polyclonal rabbit antiserum generated to the complete 53 amino acid sequence of recombinant human EGF. The RIA is highly specific for EGF (no cross reactivity with human TGF-α at 1 μmol/L).

Radioreceptor assay for TGF-α and EGF. Placentas from five fetuses of early gestation (6 to 9 weeks) and five of late gestation (37 to 42 weeks) were individually homogenized at 4° C in 5 ml PBS solution with a Polytron (Brinkmann Instruments, Westbury, N.Y.) homogenizer. Samples were centrifuged at 30,000*g* for 30 minutes at 4° C, and the supernatants were assayed for EGF receptor-active material with a modification of a radioreceptor assay described previously.[27] Briefly, confluent cultures of FaDu human squamous carcinoma cells (American Type Culture Collection, Bethesda, Md.) were washed with PBS solution and then fixed by brief exposure to 10% neutral buffered formalin for 10 minutes at room temperature. The fixed cells were washed with PBS solution containing 130 mmol/L glycine, pH 7.5, for 30 minutes to quench residual formalin, and then 100 μl of placental supernatant or standard EGF solution was added to wells along with 100 μl of iodine 125–EGF. After 2 hours of incubation at 37° C, the cells were washed three times with PBS solution and dissolved in 1 ml 4N sodium hydroxide, and the radioactivity was measured by γ-counter. Placental samples were assayed in duplicate at three dilutions and the displacement data were linearized by logit transformation. Slopes of lines generated by placental samples and EGF standard were compared for differences by *t* test for slopes. Levels of EGF receptor–active material were expressed as picograms EGF–TGF-α per milligram protein as determined by BCA protein assay.

Results

Northern blot analysis. Northern blot hybridization of polyadenylic acid–positive RNA from individual placentas obtained at various stages of gestation are shown in Fig. 1, *A*. Hybridization with the TGF-α cDNA probe generated a major band corresponding to a 4.5 kb mRNA for all the human placenta samples (lanes B through I). In addition, a similar size species was detected in polyadenylic acid–positive RNA from mouse

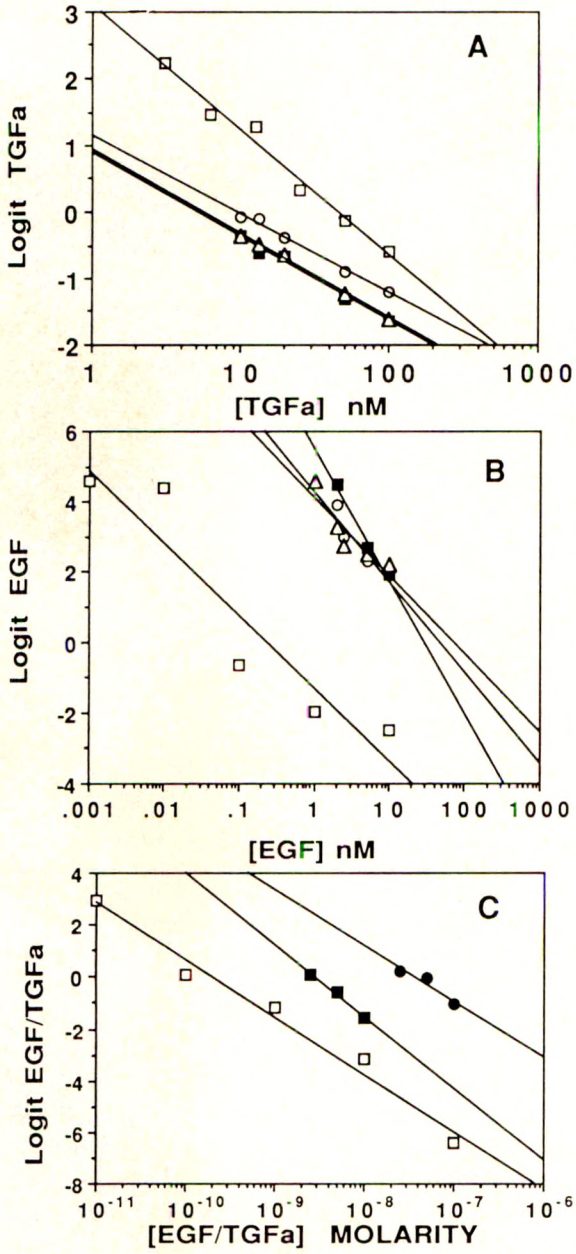


Fig. 2. Analysis of placental extracts for TGF-α and EGF immunoreactive proteins and for biologically active EGF and TGF-α proteins. Pools of human placentas from early, mid, and late gestation were extracted and analyzed for immunoreactive TGF-α **(A)** or EGF **(B)** with specific radioimmunoassays. Symbols for samples: EGF (□) or TGF-α (□) standards; early gestation (○); mid gestation (■); and late gestation (△). Analysis of placental homogenates for biologically active EGF or TGF-α proteins is shown in **C.** Homogenates of two placentas at 37 (●) and 42 (■) weeks produced displacement lines that were parallel to that generated by standard EGF (□).

decidual-placental tissue obtained at 7.5 and 11 days' gestation (lanes A and J). The size of the major hybridization band (4.5 kb) agrees with previous reports for rat TGF-α mRNA[18, 21] and human TGF-α mRNA.[23]

In addition to the major 4.5 kb species, a faint hybridization band was detected at approximately 5.4 kb in samples of 21-, 32-, and 40-week human placentas and in both mouse placenta samples. The 5.4 kb band may result from weak cross-hybridization of the TGF-α cDNA probe to EGF mRNA, which migrates at approximately the same position.

Hybridization of the same samples of polyadenylic acid–positive RNA with the EGF cDNA probe generated a faint band at 5.2 kb in all the human placentas and in both samples of mouse placenta (Fig. 1, *B*). The size of the hybridization band agreed with the size reported for prepro-EGF mRNA isolated from mouse submaxillary gland of 5.0 to 5.3 kb.[28] The intensity of the EGF cDNA hybridization signal was substantially less than that of the TGF-α cDNA hybridization signal, although the specific activity of the cDNA probes and exposure times were approximately equal.

Northern hybridization was also performed on samples of total RNA isolated from pooled placentas of early, mid, and late gestation. No hybridization bands were detected with either the TGF-α or the EGF probe, even when 10 μg of total RNA of each sample was applied to the gel (data not shown). The failure to detect hybridization bands with samples of total RNA suggests that, under the high-stringency conditions used, the TGF-α and EGF probes did not hybridize nonspecifically to RNA species present in total RNA samples and, additionally, that the levels of mRNA for TGF-α and EGF were too low to be detected by Northern analysis of total RNA samples.

RIAs for TGF-α and EGF. Detection of mRNA for TGF-α and EGF in individual placentas indicated that placental cells had the potential to synthesize TGF-α and EGF proteins. To further investigate whether the mRNA might be translated into protein, samples of placentas from early, mid, and late gestation were extracted and analyzed for TGF-α and EGF protein by specific RIAs. As shown in Fig. 2, *A*, samples of placentas from early, mid, and late gestation each generated linear displacement curves that were parallel to the TGF-α standard curve, indicating the presence of immunoreactive TGF-α protein in the samples. As shown in Table I, the levels of immunoreactive TGF-α protein measured in samples of placentas from mid or late gestation (approximately 90 ng/mg protein) were about one half the level measured in the pool of placentas from early gestation (188 ng/mg protein).

In comparison with other tissues, placenta appears to be a very rich source of TGF-α. Levels of immunoreactive TGF-α protein measured in samples of placentas from mid or late gestation were approximately fivefold higher than those measured in acid-ethanol extracts of normal and psoriatic epidermis (approxi-

mately 17 ng/mg protein),[29] approximately twentyfold higher than levels measured in chemically induced rat mammary tumors (approximately 0.5 ng/mg),[30] and approximately thirtyfold higher than levels measured in human breast cancer cell lines (3 ng/mg).[31] However, the levels of TGF-α immunoreactive protein measured in placenta or other tissues by RIA may not represent the levels of biologically active TGF-α present in the samples, because the RIA for TGF-α only detects antigenic structures present in the C-terminal 17-mer of TGF-α, which has been shown to lack the complete receptor-binding domain of intact TGF-α.[32]

Analysis of the early, mid, and late placental samples with the EGF RIA also produced linear displacement curves that were parallel to the EGF standard curve (Fig. 2, *B*). As shown in Table I, levels of immunoreactive EGF measured in the three samples from different gestational periods (between 3 and 9 pg/ml) were approximately 10,000-fold lower than the levels of TGF-α immunoreactive material. Levels of immunoreactive EGF protein have been reported for a number of other human tissues and fluids; however, these levels were usually expressed on the basis of the wet weight of tissues or the volume of fluid samples rather than on the basis of milligrams of protein in the sample. For example, the average concentration of immunoreactive EGF in cerebrospinal fluid was 233 pg/ml for men and 206 pg/ml for women.[33] Urinary levels of EGF at birth averaged 12 pg/ml (38 ng/ml creatinine), which increased during the first 2 years to approximately 70 mg/ml (170 ng/mg creatinine), then gradually decreased until puberty to approximately 82 ng/ml (70 ng/ml creatine).[34] EGF immunoreactivity has also been reported in saliva (1.9 ng/ml),[35] in seminal plasma (53 ng/ml),[36] and in milk (5 ng/ml).[37] Levels of immunoreactive EGF in homogenates of human tissues were highest in kidney (5.5 ng/gm tissue) and thyroid gland (5.3 ng/gm tissue).[38] Very high levels of EGF were found in mouse submaxillary glands (580 ng/mg protein, approximately 100,000 times higher than in human placenta), indicating that the submaxillary gland is a major site of EGF synthesis in mice.[39]

Radioreceptor assay for EGF–TGF-α. Levels of biologically active EGF and TGF-α proteins in placentas from early and late gestation were determined by radioreceptor analysis of placental homogenates. As shown in Fig. 2, *C*, supernatants of representative placental homogenates generated displacement curves that were parallel to the EGF standard curve, indicating the presence of EGF and TGF-α receptor–active protein in the samples. The average concentrations of EGF and TGF-α receptor–active protein were similar in placentas from early (246 ± 157 ng/mg protein) and late (249 ± 97 ng/mg protein) gestation. These levels of EGF and TGF-α receptor–active protein were slightly higher than the average level of immunoreactive TGF-α protein (124 ng/mg protein) measured in placentas from early, mid, and late gestation.

Table I. Levels of immunoreactive TGF-α and EGF protein in pools of human placentas from early, mid, and late gestation

Stage of gestation	*TGF-α (ng/mg)*	*EGF (pg/mg)*
Early	189 ± 28	8 ± 3
Mid	93 ± 13	3 ± 1
Late	91 ± 12	9 ± 2

Placentas from different stages of gestation were pooled, extracted, and analyzed for EGF or TGF-α proteins with specific RIAs. All placental samples generated displacement curves with slopes parallel to EGF or TGF-α standard curves. Values are mean ± SE of concentrations determined from logit transformation of multiple dilutions of samples and are expressed as nanogram equivalents of EGF or TGF-α per milligram of soluble protein.

Comment

Previous reports have implicated TGF-α, EGF, and their receptors in the development and functioning of the placenta in vivo. These include the expression of the TGF-α–EGF receptor by placental cells[5-7] and EGF stimulation of secretion of placental peptide and steroid hormones by cultures of placental cells in vitro.[8-11] However, the source of TGF-α or EGF that could influence placental cell function has not been established. Circulating plasma levels of EGF and TGF-α appear to be too low to significantly influence placental cells[13-16]; therefore production of the factors by placental cells is a more likely source.

Our results demonstrate the presence of substantial levels of TGF-α mRNA and high levels of immunoreactive and receptor-active TGF-α protein in extracts of human placental tissue throughout gestation. These results support the concept that TGF-α may act by an autocrine or paracrine mechanism to influence human placental cells. Additional studies are needed, however, to determine if the TGF-α and EGF mRNA is translated into protein and which cells in the maternal-fetal-placental unit synthesize the proteins before it can be conclusively determined that these peptides influence placental cells by autocrine or paracrine mechanisms.

Although human placenta has not been analyzed previously for TGF-α mRNA and immunoreactive protein throughout gestation, human term placenta was reported to contain biologic activity consistent with either EGF or TGF-α. Stromberg et al.[40] reported that acid-ethanol extracts of term human placentas contained four different size species (6, 10, 20, and 30 kd) that stimulated growth of NRK cells in soft agar and competed for binding of ^{125}I-EGF to EGF–TGF-α membrane receptors. Levels of EGF and TGF-α receptor–

binding activity were in the range of 11 ng of EGF equivalents per placenta. Interestingly, analysis of the 6 and 10 kd samples for EGF by a specific human RIA failed to detect any immunoreactive EGF material. Thus their results generally agreed with our finding of low levels of immunoreactive EGF protein.

In a study of mouse embryos, Nexo et al.[41] detected low levels of immunoreactive EGF in extracts of 15-day embryos (approximately 1 ng/gm wet weight) that were approximately fortyfold lower than the levels of EGF and TGF-α receptor–active material detected in 12- to 17-day mouse embryos. Popliker et al.,[42] however, reported no detectable levels of EGF mRNA in mouse fetuses or placenta from day 9 of gestation through birth. They reported that the onset of endogenous synthesis of EGF began in neonatal mice in the kidney at 2 weeks post partum. These results lead them to conclude that immunoreactive EGF protein and EGF receptor–active protein detected in mouse fetus and placenta were probably of maternal origin. Taken together, these series of studies[40-42] suggest that placental tissue from mouse, rat, and human contains little or no immunoreactive EGF protein but does contain substantial levels of EGF and TGF-α receptor–active protein(s). On the basis of our analysis of human placentas for EGF and TGF-α mRNA, immunoreactive proteins, and receptor–active proteins, the paradoxic results reported previously of low levels of immunoreactive EGF protein but high levels of EGF and TGF-α receptor–active proteins may be due to placental synthesis of TGF-α protein that was not detected by EGF RIAs but was detected by EGF–TGF-α radioreceptor assays.

Our results do not establish which cells within the human placenta synthesize TGF-α mRNA or which cells contain TGF-α protein. In situ hybridization studies with cDNA probes specific for TGF-α could indicate which placental cells contain TGF-α mRNA, and immunohistochemical analysis with antibodies specific for TGF-α could designate which cells contain immunoreactive TGF-α. Han et al.[18] used in situ hybridization and Northern blot analysis to demonstrate that maternal decidual cells of 8-day rat fetuses were the primary source of TGF-α mRNA, not embryo cells. At 14 days of gestation, however, they detected low levels of TGF-α mRNA in rat placental cells. Human uterine decidual cells have not been examined for TGF-α mRNA or protein, and particular care was taken to exclude any maternal decidual cells or uterine cells in the samples of human placentas. If human decidual cells synthesize and secrete TGF-α or EGF, it is possible that the peptides could influence placental syncytiotrophoblasts through a paracrine mechanism.

The substantial levels of TGF-α mRNA and protein detected in human placenta throughout gestation provide evidence that supports the concept that TGF-α and its receptor may influence normal human fetal placental development and function. Additional studies are needed, however, to begin to understand what roles TGF-α and its receptor play in normal and abnormal human placental function. Insight might be gained by determining if levels of TGF-α mRNA and protein are altered significantly during abnormal placental development. In this initial study of normal placentas, Northern analysis of polyadenylic acid–positive RNA from eight individual human placentas suggested that TGF-α mRNA levels were similar throughout gestation and immunoreactive TGF-α protein levels were similar in pools of placentas from early, mid, and late gestation. Further studies will be required to accurately assess whether TGF-α mRNA and protein levels change significantly during normal and abnormal placental development, but the apparent autocrine action of TGF-α in human placenta suggests that TGF-α and its receptor may play key roles in placental physiologic characteristics.

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Oxytocin given in a pulsatile manner to the ewe at 120 to 140 days' gestational age increases fetal sheep plasma cortisol

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A single intravenous injection of oxytocin into pregnant sheep (123 to 144 days' gestational age) causes a bout of myometrial activity and an increase in fetal plasma corticotropin levels. We hypothesized that a sustained increased frequency of myometrial contractures accelerates the normal increase in fetal adrenal secretion in sheep in the last 3 weeks of gestation. To test this hypothesis, pulses of saline solution (group 1, 9 ewes and 10 fetuses) or oxytocin (group 2, 11 ewes and 12 fetuses) 600 or 960 μU/kg/min for 5 of every 30 minutes were infused into the maternal jugular vein for 6 days beginning at day 128 ± 2 (mean ± SD) days' gestational age. Total myometrial activity increased to 160% of baseline in group 2 by day 6. Myometrial activity did not change throughout the study in group 1. Maternal plasma cortisol concentrations did not rise during oxytocin infusion when compared with their own baselines (group 2) and were not different from concentrations in saline-infused ewes (group 1). By day 4 of oxytocin administration fetal plasma cortisol concentrations had risen significantly above baseline in group 2 ($p < 0.05$). Fetal plasma cortisol concentrations did not rise in group 1. Corticotropin levels were not elevated in the fetal carotid arterial plasma of either group. A small but significant decrease occurred in fetal carotid arterial Po_2 in group 2 by day 6 but not in group 1. In conclusion, increased myometrial activity produced by pulsed oxytocin is accompanied by increased fetal plasma cortisol concentrations, demonstrating that long-term alteration of myometrial activity affects fetal adrenal function over several days at this critical period of gestation. (Am J Obstet Gynecol 1992;166:200-5.)

Key words: Pulsatile infusion, oxytocin, fetal cortisol, myometrial contractility, fetal sheep

Spontaneous myometrial activity occurs throughout the second half of pregnancy in many species, including sheep,[1] rhesus monkey,[2] and human beings.[3] Epochs of myometrial activity of prolonged duration (3 to 15 minutes) are called contractures and are the predominant form of activity until the onset of labor, when they are replaced by short-lived, expulsive contractions. Contractures are time-associated with various physical stimuli to the fetus: decrease in fetal arterial Po_2[4]; deformation of the fetus, as seen as a decrease in the dorsal-ventral and an increase in the transverse dimensions of the chest wall diameter[1]; increase in intracranial pressure[5]; and a redistribution of fetal blood to the carcass away from the placenta.[6] Oxytocin-induced contractures have been shown to stimulate fetal corticotropin secretion.[7] At 123 to 144 days' gestational age a 100 mU oxytocin bolus given to a pregnant ewe caused a single myometrial electromyographic epoch of activity and an elevation in fetal corticotropin levels to 145% of baseline. Corticotropin levels remained elevated for 20 minutes.[7]

Ovine fetal plasma cortisol begins to rise slowly from 10 to 40 pg/ml over the last 20 days of pregnancy.[8] Maturation of the fetal hypothalamic-pituitary-adrenocortical axis plays an important role in the initiation of parturition as well as in the maturation of many fetal organ systems. We hypothesize that a sustained increase in the frequency of myometrial contractures in the last 3 weeks of gestation will alter fetal adrenocortical function in the sheep. We elected to increase myometrial activity with a regimen of 5-minute pulses of oxytocin administered intravascularly to the ewe every 30 minutes because this regimen is effective in increasing contracture frequency for several days without the loss in efficacy in stimulating myometrial activity that occurs when oxytocin is continuously infused.[9]

Material and methods

Care of animals. Twenty Rambouillet and Dorset cross ewes of known gestational age were used in these studies. Two ewes, one from each group, had twin fetuses. Animals were housed in metabolic cages and given free access to alfalfa cubes and water ad libitum. Lights were on for 16 hours each day beginning at

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6 AM. New York State College of Veterinary Medicine guidelines for the care and use of the sheep were followed. Experiments were conducted under an approved Cornell Institutional Animal Care and Use Committee protocol.

Before surgery animals were divided into two groups: group 1 (saline solution control; 9 ewes, 10 fetuses) and group 2 (oxytocin-infused; 11 ewes, 12 fetuses). Surgery was conducted with halothane general anesthesia at 118 ± 2 days' gestational age (mean ± SD) with techniques described previously.[1] Ewes were instrumented with polyvinyl catheters placed in the jugular vein and carotid artery and with multistrand bipolar stainless steel wires (AS632; Cooner Sales Co., Inc., Chatworth, Calif.) sewn 5 mm apart into the myometrium of the pregnant horn to record a myometrial electromyogram. Each fetus was instrumented with polyvinyl catheters inserted into the jugular vein and carotid artery, stainless steel wires terminating in metal pins inserted bilaterally through the frontal bone into the dura to record the fetal electroencephalogram, and electrode wires sewn into the fascia above and below both eyes to record the fetal electrooculogram and sewn into the deep nuchal antigravity muscles of the neck.[1] Fetal electrophysiologic data are not reported here.

Oxytocin infusion. Both groups of sheep were allowed a 7-day period for recovery from surgery and a further 2-day period for collection of baseline myometrial electromyography and plasma samples for hormone measurement. The experimental period began immediately after the baseline period. In group 2, pulses of oxytocin (Anthony Products, Arcadia, Calif.) were infused into the maternal jugular vein beginning at 128 ± 2 days' gestational age (day 1) at a rate of either 900 (n = 5) or 600 (n = 7) μU/kg/min (1.9 or 1.2 ng/kg/min) in physiologic saline solution for 5 of every 30 minutes. Fetuses in the control group (group 1) received saline solution at the same flow rate and pulse regimen beginning at 127 ± 1 days' gestational age. Infusions were continued for 6 days.

Blood sampling. Maternal and fetal blood samples were drawn into sterile chilled syringes and centrifuged at 4° C; plasma was immediately flash frozen in liquid nitrogen. Fetal and maternal blood gases were determined with 0.5 ml blood in an ABL2 Acid Base Laboratory (c version; Radiometer A/S, Copenhagen). Maternal and fetal plasma (0.5 ml) for measurement of cortisol concentrations was taken twice daily before the start of a pulse, but not during a contracture. Sampling was possible in one animal for 5 days of the experimental period; all other animals were sampled for 6 days of the experimental period for cortisol measurement. In 17 sheep from the two groups a frequent sampling protocol was carried out to overcome potential problems of pulsatile fetal corticotropin secretion. Plasma (0.7 ml) for measurement of fetal corticotropin levels was taken every 5 minutes for a 60-minute period at 4 PM ± 2 hours (mean ± SD) on 1 baseline day and on another day during the periods 1 to 4 and 5 to 8 days after start of pulsatile infusion. This resulted in 12 samples from 1 day. Fetal blood was handled aseptically and the erythrocytes returned at the end of the day's sampling.

Cortisol radioimmunoassay. Plasma cortisol concentration was measured (Autopak cortisol kit, Micromedic Systems Inc., Horsham, Pa.) as previously described.[10] Twenty microliters of plasma was extracted with the dichloromethane method[11] and assayed in antibody-coated tubes (antibody raised against histamine-3-cortisol). Maternal plasma extraction efficiency is slightly higher (89%, n = 24) than fetal (86%, n = 24). Values obtained from the assay were corrected for recovery. Intraassay coefficients of variation were 9.0% for the maternal pool (mean concentration 68.7 ng/ml, n = 24) and 10.0% for the fetal pool (mean concentration 13.3 ng/ml, n = 10). With a different set of pools, the interassay coefficient of variation was 18.3% for the maternal pool (mean concentration 45.5 ng/ml, n = 9) and 22.6% for the fetal pool (mean concentration 19.5 ng/ml, n = 9). The range of the standard curve was 0.96 to 64 ng/ml for the plasma samples. Assay sensitivity, calculated as the upper value of 95% confidence interval of bound/unbound, was 0.9 ng/ml of sample.

Immunoreactive corticotropin radioimmunoassay. Plasma corticotropin was measured (INC Star corticotropin kit, Stillwater, Minn.). The method we described previously was miniaturized.[12] Plasma was thawed on ice and 50 μl plasma was incubated with rabbit antihuman corticotropin (fragment 1-24) antiserum for 24 hours at 4° C, and then incubated with iodinated corticotropin (fragment 1-24) for another 24 hours at 4° C. The bound fraction was precipitated with a goat antirabbit second antibody. Interassay coefficients of variation for fetal pool (mean concentration 28.8 pg/ml, n = 9) were 12.5% and for maternal pool (mean concentration 42.8 pg/ml, n = 8) were 8.2%. Intraassay coefficients of variation for fetal and maternal pools were 6.1% and 3.0% respectively. Assay sensitivity, calculated as the 90% bound/unbound limit of detection was 5.0 pg/ml of sample.

Myometrial electromyogram, data acquisition, and analysis. The recording of myometrial electromyographic activity was performed continuously for 8 days, integrated over 8-second intervals, and analyzed as previously described.[13] Twenty-four–hour analysis periods commenced with the first oxytocin or saline solution pulse at 4 PM ± 2 hours (mean ± SD). Total myometrial electromyographic activity is the algebraic sum of electromyogram peak height (voltage) at each 8-second interval during the 24-hour period.

Statistical analysis. Myometrial electromyogram val-

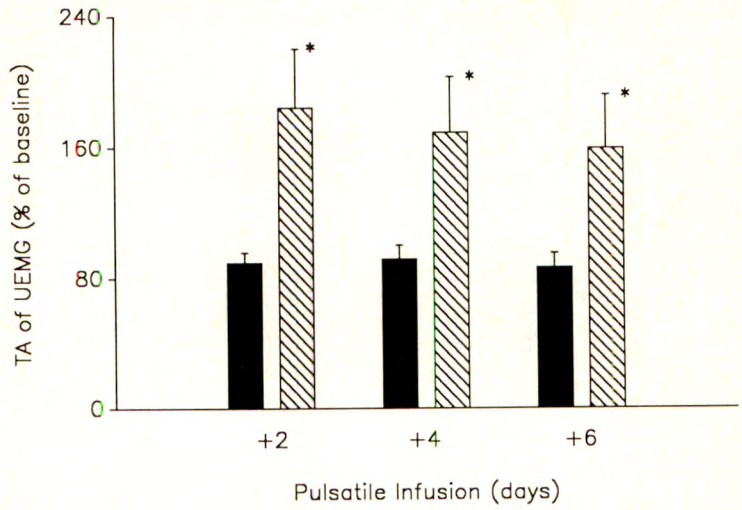


Fig. 1. Total activity *(TA)* of myometrial electromyogram *(UEMG)* (mean ± SEM) as percent of baseline in control fetuses (group 1, $n = 8$, *solid bar*) and fetuses of oxytocin-pulsed ewes (group 2, $n = 6$, *striped bar*) on days 2, 4, and 6 after the start of the pulsatile infusion regimen. Split plot analysis was performed with a set of orthogonal contrasts to compare values in the two groups at each time point. *Asterisk*, $p < 0.05$.

ues were normalized and are presented in the form of percentage of the mean on the 2-day baseline period (experimental day activity/baseline activity × 100). For example, 160% of baseline represents a 60% increase above baseline. Fetal plasma cortisol concentrations were log transformed for analysis. Split plot analysis was performed with a set of orthogonal contrasts to compare values at each time point. Significance is given at $p < 0.05$ level. Data throughout are given as mean ± SEM.

A one-sided t test (where group 2 > group 1 was expected) of fetal plasma corticotropin values was performed with the mean of the 12 samples in each series, the coefficient of variation, and the maximum and minimum corticotropin value observed in each 12-sample series. Groups 1 and 2 were compared at baseline and at 1 other day during the periods of 1 to 4 and 5 to 8 days after the start of pulsatile infusion. Significance is given at $p < 0.05$ level.

Results

Blood gases. Fetal carotid arterial Po_2 was 22.6 ± 0.6 mm Hg in group 1 and 20.0 ± 0.9 mm Hg in group 2 over the 3 to 6 days before commencement of infusion. By day 6 of infusion, fetal Po_2 remained unchanged in group 1 (21.9 ± 1.1 mm Hg) and had fallen significantly in group 2 (18.0 ± 0.9 mm Hg), $p < 0.05$. Maternal blood gases on the baseline day were Po_2 99.6 ± 5.1 mm Hg and pH 7.47 ± 0.01 for group 1 and Po_2 102.8 ± 4.0 mm Hg and pH 7.45 ± 0.02 for group 2; these did not change throughout the study. The blood gas analyzer was not operational at some periods; thus complete data were available for analysis from only seven fetuses in group 1 and eight fetuses in group 2.

Myometrial electromyographic data. Total myometrial electromyographic activity was increased ($p < 0.05$) in group 2 ($n = 6$; the electromyograms of two individual animal preparations were monitored continuously on a polygraph to confirm increased activity, but they were not stored in the computer for analysis) for all days as compared with the corresponding days in group 1 ($n = 8$) (Fig. 1). Data in this figure are normalized to baseline values equal to 100%. The duration of individual contractures did not change throughout the study in either group 1 or 2. In group 1 contractures lasted 391.8 ± 59.3 seconds before saline solution pulses and 375.4 ± 69.4 seconds on day 4 of pulsatile saline solution administration. In group 2 contractures lasted 359 ± 59.0 seconds before oxytocin pulses were started and 333.3 ± 65.8 seconds on day 4 of oxytocin administration.

Cortisol. Maternal carotid arterial plasma cortisol concentrations were not different between the two groups during the baseline period (group 1, 10.0 ± 1.8 ng/ml, group 2, 5.9 ± 0.8 ng/ml) and did not change during the experimental period. On day +6 maternal plasma cortisol for group 1 was 6.8 ± 1.6 ng/ml, and for group 2 was 5.3 ± 0.8 ng/ml. Fetal carotid plasma cortisol concentrations were significantly elevated in group 2 by days 4 and 6 ($p < 0.05$) (Fig. 2). Baseline and day 6 cortisol values for group 1 fetuses were 4.4 ± 1.3 and 6.1 ± 1.7 ng/ml, respectively, and those for group 2 fetuses were 4.5 ± 1.6 and 12.5 ± 2.3 ng/ml, respectively ($p < 0.05$). Ten individual fetal preparations were used for group 1 except for the day

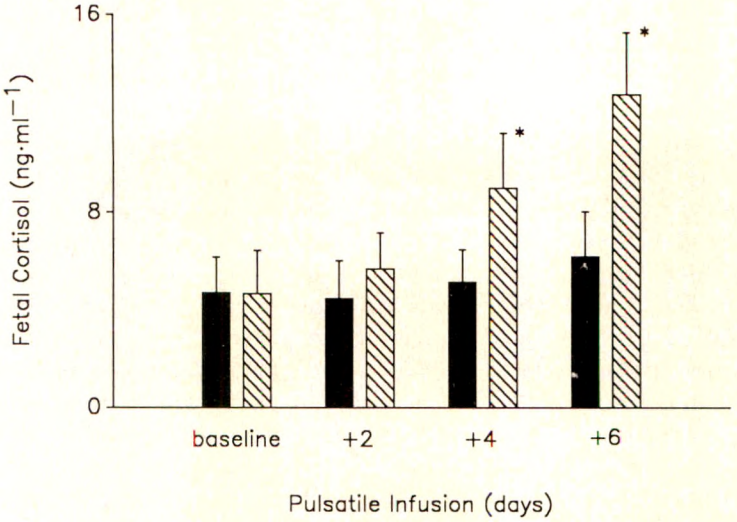


Fig. 2. Fetal arterial plasma cortisol concentrations (mean ± SEM) in control fetuses (group 1, $n = 10$, *solid bar*) and fetuses of oxytocin-pulsed ewes (group 2, $n = 12$, *striped bar*) on baseline and days 2, 4, and 6 after the start of the pulsatile infusion regimen. Split plot analysis was performed with a set of orthogonal contrasts to compare values in the two groups at each time point. *Asterisk*, $p < 0.05$.

Table I. Analysis of fetal arterial plasma corticotropin concentrations

Group	*Days of infusion*	*Fetal plasma corticotropin values (pg/ml)*	*Coefficient of variation (%)*	*Maximum fetal plasma corticotropin values (pg/ml)*	*Minimum fetal plasma corticotropin values (pg/ml)*
Saline ($n = 7$)	Baseline	13.2 ± 2.2	39.9 ± 19.1	20.3 ± 2.5	9.2 ± 2.2
Oxytocin ($n = 10$)		14.4 ± 2.6	47.5 ± 18.6	20.6 ± 3.3	9.8 ± 2.1
Saline	1-4	19.8 ± 3.9	22.0 ± 7.1	32.3 ± 11.1	14.1 ± 2.0
Oxytocin		18.8 ± 3.0	39.4 ± 11.7	28.5 ± 4.0	12.3 ± 2.5
Saline	5-8	19.5 ± 3.9	19.8 ± 4.1	26.8 ± 5.1	14.8 ± 3.6
Oxytocin		23.5 ± 4.6	31.7 ± 8.0	35.2 ± 8.6	13.8 ± 3.8

Twelve samples were taken at 5-minute intervals from each fetus on a baseline day and one other day during the periods 1 to 4 and 5 to 8 days after start of the pulsatile infusion regimen. Values are mean ± SEM. There were no significant differences between groups.

6 sample, in which one fetus's samples were not obtained. Twelve individual fetal preparations were used for group 2 except for the day 4 and 6 samples, in which one fetus's samples were not obtained.

Corticotropin. Table I shows the mean fetal plasma corticotropin, coefficient of variation, and maximal and minimal values in both groups during the 1-hour frequent sampling bout on the baseline day, 1 day in the 1- to 4-day window, and 1 day in the 5- to 8-day window after the start of pulses. None of the fetal carotid plasma corticotropin variables were different between the two groups at any of the time periods.

Comment

Our intention in choosing the oxytocin-infusion regimen was to increase myometrial activity only to the higher end of the range normally seen in pregnant sheep, thus keeping our investigation of fetal adrenal function within physiologic limits. Myometrial electromyographic activity was increased to 160% of baseline in group 2. The pulsatile oxytocin infusion regimen used in group 2 delivered a lower amount of oxytocin to the ewe than the regimens used by other investigators.[7] Oxytocin infusion (960 μU/kg/min) resulted in a peak maternal uterine vein oxytocin plasma concentration of 70 pg/ml at the end of the 5-minute pulse. Maternal uterine vein plasma oxytocin concentration was back to baseline (20 pg/ml) by 15 minutes from the start of the pulse.[9] Because oxytocin does not cross the ovine placenta,[14] oxytocin's effect on fetal cortisol concentrations must be produced indirectly via changes in the ewe or placenta.

Our study was designed to test the hypothesis that increased myometrial activity would result in evidence

of increased fetal adrenal activity. The increased fetal plasma cortisol concentrations may have been caused by one or more of a variety of intermediary mechanisms. We and others have shown that myometrial contractures are associated with both increased sensory stimulation to the fetus[1] and decreased uterine blood flow, which leads to a fall in fetal arterial P_{O_2}.[4] The alterations of fetal chest wall diameters that take place during contractures show that the fetus is deformed to a varying extent by individual contractures.[1] This influence on the fetus should be accentuated by the increase in total uterine contractility produced by oxytocin administration in group 2. Although the fetal P_{O_2} decreased in group 2 fetuses, this level of arterial P_{O_2} would still be considered healthy, suggesting that the increased uterine contractility did not compromise the fetus. It has been shown that mild levels of fetal hypoxia slightly greater than those seen here (a decrease of <5 mm Hg from baseline) maintained for 24 hours were accompanied by elevated plasma cortisol values in fetal sheep (122 to 139 days' gestational age).[15] Further studies in which the effects of contractures on fetal shape and oxygenation are controlled during oxytocin administration will be necessary to determine which of these two stimulatory pathways are involved.

Fetal plasma cortisol levels show considerable variation over a 90-minute period.[16] Our study samples were taken with the intent of minimizing variation related to myometrial activity by avoiding periods of myometrial activity and sampling just before administration of an oxytocin pulse. The increase in fetal plasma cortisol concentrations observed in the group 2 fetuses resembles the beginning of the normal predelivery rise seen at about 136 to 140 days' gestational age.[8] Fetal plasma cortisol concentrations in both groups are considerably lower than concentrations seen immediately before spontaneous[8] or corticotropin-induced[17] parturition, and also less than those seen in fetuses of the same gestational age in conditions of a 2-hour hypoxemic[18] or 10-minute hypotensive[19] stress. Thus the changes in fetal cortisol seen on day 4 and day 6 of oxytocin administration were within the physiologic range.

We were unable to show an increase in mean fetal plasma immunoreactive corticotropin levels accompanying the increased fetal plasma cortisol concentrations in the fetuses of oxytocin-infused ewes. Nor could we show a change in fetal release of corticotropin based on coefficient of variation of each series or maximum and minimum values. These indices reflect the overall variability and range of this variability under each treatment. It is possible that the oxytocin-induced contractures influence fetal adrenal cortisol secretion via nonpituitary mechanisms. We have shown that bilateral splanchnic nerve section of the fetal lamb in late gestation will significantly lower the rise in fetal plasma cortisol concentrations in response to hypotension in the absence of any change in the rise in fetal plasma corticotropin.[20] Thus sensory stimulation of the fetus by contractures may affect neural mechanisms that influence adrenocorticol and medullary function via the splanchnic nerves. It is now apparent that interrelationships exist between the adrenal cortex and the medulla.[21] Other potential mechanisms for stimulation of the increased fetal plasma cortisol we observed are increased prostaglandin production,[22] corticotropin from the placenta,[23, 24] or another source that is bioactive but not immunoreactive in our assay. Prostaglandin E_2 has been implicated in the stimulation of a rise in fetal adrenal cortisol production directly without a concomitant rise in corticotropin.[22]

In conclusion, these data show that fetal plasma cortisol concentration in late gestation is elevated in response to increased myometrial activity associated with a small but statistically significant fall in fetal carotid arterial P_{O_2}. Because the increase in total myometrial contractility was within physiologic limits, these results are compatible with the view that the fetus responds to signals from the mother that may act through altered myometrial activity. These findings also have implications in physiologic and clinical conditions in which the extent of stimulation of contractures is altered as in oligohydramnios or polyhydramnios.

We thank Karen Moore for her excellent assistance with this manuscript.

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Editors' note

The American Journal of Obstetrics and Gynecology introduces a new format for abstracts accompanying regular articles, society articles, and Current Investigation articles. Authors submitting these manuscripts to the Journal should provide an abstract of no more than 150 words structured according to the following headings: Objective(s), Study Design, Results, and Conclusion(s). Exceptions to this requirement include Clinical Opinion, Current Development, case report, and brief communication articles. Abstracts for these articles will continue to follow the standard abstract format. Please consult the Information for Authors for details.

Both 17β-estradiol and tamoxifen induce c-*fos* messenger ribonucleic acid expression in human endometrial carcinoma grown in nude mice

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We previously demonstrated the estrogen-like effects of tamoxifen on the acceleration of growth and increased progesterone receptor concentrations of human endometrial carcinomas grown in the nude mouse experimental model. In our current study the modulation of protooncogene expression by 17β-estradiol and tamoxifen in human endometrial carcinomas was investigated. The protooncogenes investigated in this study were c-*fos*, c-*jun*, c-*myc*, N-*myc*, HER-2/*neu*, c-*erb*B, c-*fms*, and c-Ha-*ras*. Among those we found that c-*fos* expression was induced by 17β-estradiol in the following 17β-estradiol–sensitive tumors: EnCa-101 and EnCa-X. The induction was apparent within 1 hour, reached peak level at 2 hours (16-fold), and remained constant up to 4 hours. The c-*fos* messenger ribonucleic acid returned to prestimulation level by 12 hours. Tamoxifen also stimulated c-*fos* expression, the expression pattern being similar to that of 17β-estradiol albeit of a lesser degree. The messenger ribonucleic acid transcripts for other protooncogenes tested did not show significant changes during hormonal manipulation. The induction of c-*fos* expression by tamoxifen is consistent with its estrogen-like effect on endometrial carcinoma growth. (Am J Obstet Gynecol 1992;166:206-12.)

Key words: Tamoxifen, estradiol, endometrial carcinoma, protooncogenes, mRNA expression

A series of human endometrial carcinomas displaying different degrees of steroid hormone responsiveness in the nude mouse model has been established.[1, 2] Two of these established, well-differentiated endometrial carcinomas, EnCa-101 and EnCa-X, are steroid receptor positive and responsive to 17β-estradiol (E_2) and progesterone. As observed in normal endometrium E_2 stimulates growth and increases progesterone receptor, and progesterone inhibits growth and induces secretory changes in these tumors.[3, 4] Tamoxifen, a synthetic triphenylethylene compound, exhibited E_2-like effects in these tumors with regard to increased growth and progesterone receptor concentration.[3, 5] The paradoxic effect of tamoxifen on endometrial carcinoma was in contrast to its E_2-antagonistic effect on human breast carcinoma.[6]

The transient expression of immediate, early genes (protooncogenes) was widely reported to be related to cell proliferation-differentiation.[7] Modulation of expression of c-*fos*, c-*myc*, N-*myc*, c-Ha-*ras*, c-*erb*B, and c-*jun* by E_2 has been demonstrated in rodent uterus, chick oviduct, and human breast carcinoma cells in culture.[8-15] To ascertain whether the growth-stimulatory effect of tamoxifen on endometrial carcinoma was reflected in its effects on these immediate, early genes we studied the effects of E_2 and tamoxifen on the expression of various protooncogenes in EnCa-101. The effects of E_2 and tamoxifen in an E_2-insensitive, poorly differentiated endometrial carcinoma, EnCa-138, were compared in parallel.

Material and methods

Materials. The following materials were used: E_2, tamoxifen, and placebo pellets (Innovative Research of America, Toledo); α-phosphorus 32 2′-deoxycytidine-5′-triphosphate (dCTP) (Amersham, Inc., Arlington Heights, Ill.), guanidinium thiocyanate, oligo dT-cellulose, and random-primed deoxyribonucleic acid (DNA)–labeling kit (Boehringer Mannheim Biochemical, Indianapolis); sodium acetate, sodium chloride, dextran sulfate sodium salt, sodium dodecyl sulfate (SDS), sodium citrate, and salmon sperm DNA (Sigma Chemical Co., St. Louis); cesium chloride, formamide, and restriction endonucleases (Bethesda Research Laboratories, Gaithersburg, Md.); nylon membranes (Schleicher and Schuell, Inc., Keene, N.H.); oncogene probes v-*fos* (p*fos*-1), N-*myc* (pNB-1), HER-2/*neu* (pHER-2-436-1), c-Ha-*ras* (*pbc*-N1), v-*fms* (pSM7C), and

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v-*erb*B (pAE*Bam*-R1) (American Type Culture Collection, Rockville, Md.); complementary DNA (cDNA) clone pHJ40 (c-*jun*) (Dr. Robert Tjian, University of California, Berkeley); and β-actin cDNA (Dr. George Oyler, The Pennsylvania State University).

Animals. Four- to five-week-old male BALB/c, nu/nu nude mice (Harlan Sprague Dawley, Inc., Indianapolis) were maintained in separate barrier facilities used exclusively for these animals. They were castrated at least a week before use.

Tumor transplantation and growth. The E_2- and tamoxifen-sensitive tumors (EnCa-101 and EnCa-X) and insensitive tumor (EnCa-138) were derived from primary carcinomas of human endometrium by serial transplantation in the nude mouse model.[2] EnCa-101 is a histologically well-differentiated adenocarcinoma, and EnCa-138 is a poorly differentiated adenosquamous carcinoma. Tumor tissues (~20 mg) were transplanted subcutaneously in the infrascapular and lumbar regions at four sites of 4- to 6-week-old, castrated, athymic BALB/c, nu/nu nude mice. The animals were divided into three groups (three mice per group): group 1, control pellets (cholesterol, methylcellulose, lactose matrix); group 2, E_2 pellets (blood level maintained at 200 pg/ml for 60 days); group 3, tamoxifen pellets (blood level maintained at 20 to 30 ng/ml for 60 days). These pellets were implanted into mice subcutaneously. Growth rate of the tumors was monitored by determining tumor volume with a vernier calipers at weekly intervals.

Protooncogene messenger ribonucleic acid (mRNA) expression. For protooncogene mRNA expression studies, the tumors were grown without implants. When the tumors reached 8 mm in geometric mean diameter, the animals were implanted with E_2 or tamoxifen pellets subcutaneously (n = 4). The tumors were removed at 0, 0.5, 1, 2, 4, 6, 12, and 24 hours thereafter. Three separate experiments were carried out with essentially similar results.

Northern blot analysis. The tumors were pulverized and stored in liquid nitrogen immediately after removal. Ribonucleic acid was prepared as described by Chirgwin et al.[16] Briefly, pulverized tumor samples were homogenized in a solution containing 4 mol/L guanidinium thiocyanate and 25 mmol/L sodium acetate (pH 6.0) (Polytron, Brinkmann Instrument Co., Westbury, N.Y.). After ultracentrifugation through a dense cushion of cesium chloride, total RNA was precipitated by ethanol. Polyadenylic acid [poly(A)]–positive RNA was isolated by affinity chromatography with oligo(dT) cellulose.[17] Two to five micrograms of poly(A)–positive RNA was denatured at 95° C for 2 minutes, fractionated in 1% agarose gels containing 0.66 mol/L formaldehyde, and transferred to nylon membranes in 10× SSC (1.5 mol/L sodium chloride, 0.15 mol/L sodium citrate dehydrate). Nylon membranes were baked at 80° C for 2 hours and prehybridized for 3 to 12 hours at 42° C in a solution containing 50% deionized formamide, 1% SDS, 1 mol/L sodium chloride, and 10% dextran sulfate. Thereafter hybridization was performed for 16 to 18 hours at 42° C in the prehybridization buffer containing sonicated and denatured salmon sperm DNA (50 μg/ml) and 1.0 × 10^6 cpm ^{32}P-labeled DNA probe per milliliter (specific activity 1 to 2 × 10^9 cpm/μg DNA). The DNA probes were labeled with α-^{32}P dCTP with random-primed DNA-labeling kit. After hybridization, nylon membranes were washed as follows: twice in 2× SSC at room temperature for 5 minutes, twice in 2× SSC and 1.0% SDS at 65° C for 30 minutes, and twice in 0.1× SSC at room temperature for 30 minutes.

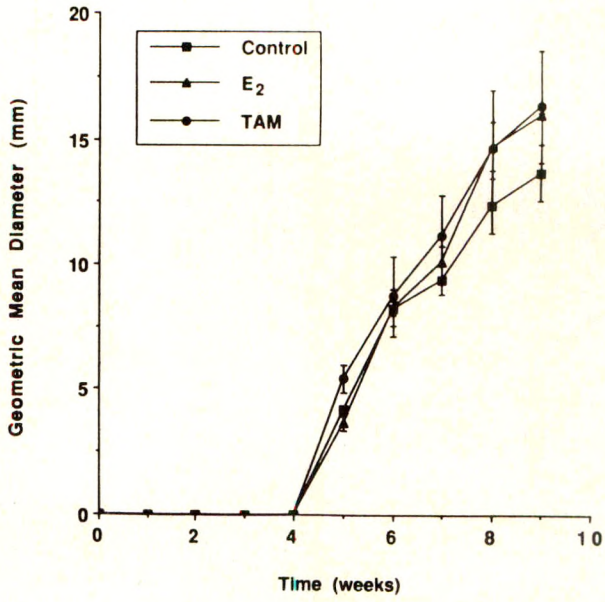


Fig. 1. Tumor-growth study of poorly differentiated EnCa-138 endometrial carcinoma. Animals bearing EnCa-101 tumors received placebo, E_2, 1.7 mg or tamoxifen (*TAM*, 5 mg) pellets. Growth rate of the tumors was monitored weekly by determining tumor size with vernier calipers. Results were expressed as mean; *bars*, SD (control, n = 12; tamoxifen, n = 12; E_2, n = 8).

The nylon membranes were exposed to film (Kodak X-AR5, Eastman Kodak, Rochester, N.Y.). The probe was removed by washing the membranes with 0.01× SSC and 0.01% SDS at 95° C several times for a few minutes. Autoradiography was performed to verify removal of the probe, and the membranes were hybridized to a second probe. Autoradiograms were scanned by a laser densitometer (Protein Databases, Inc., Huntington Station, N.Y.) to determine relative levels of protooncogene expression.

Radioimmunoassay of serum E_2. Male BALB/c mice (n = 22) were castrated 1 week before the study. Eigh-

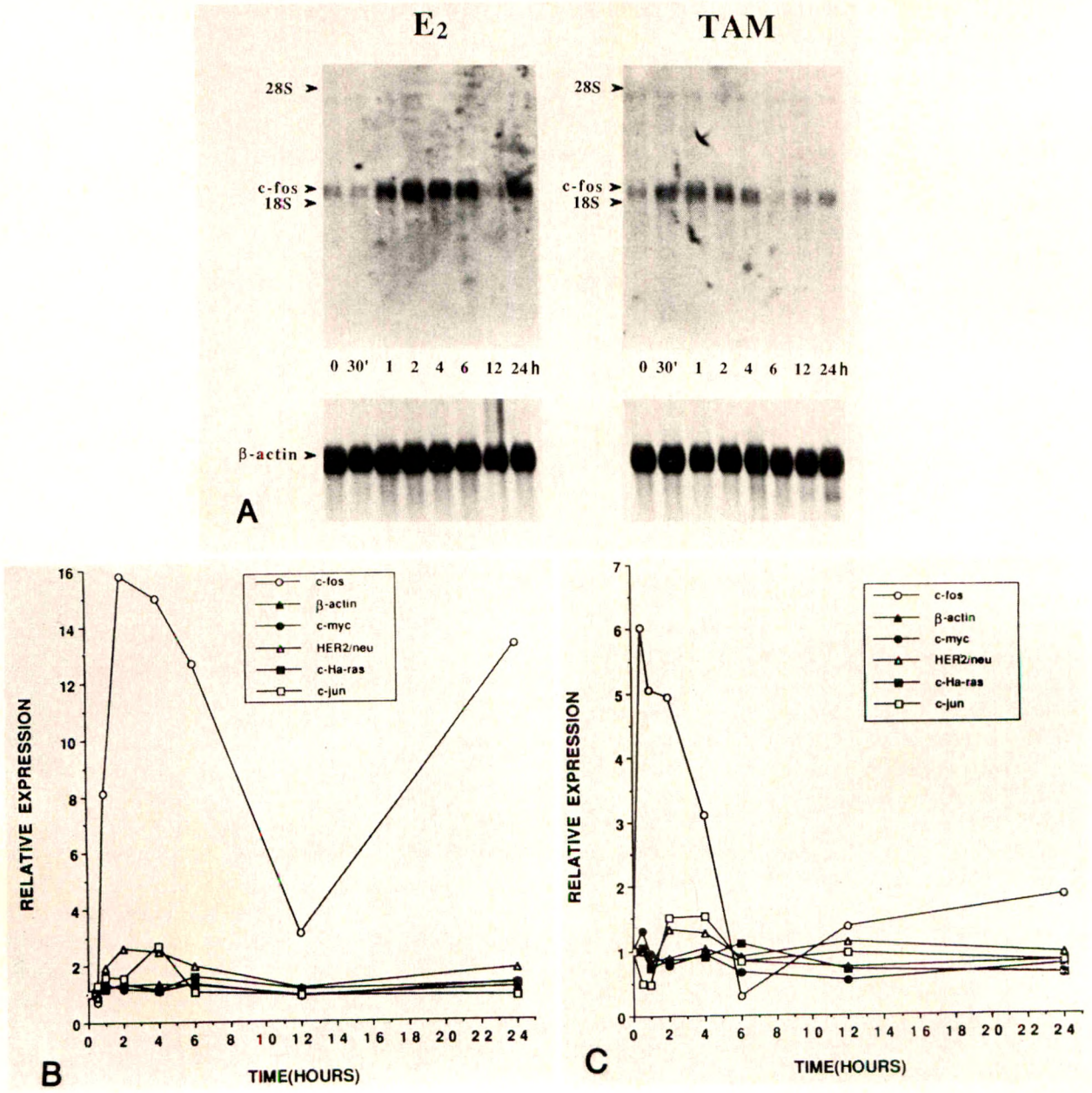


Fig. 2. A, Northern blot analysis of c-*fos* mRNA expression by E_2 and tamoxifen *(TAM)* in EnCa-101. Well-differentiated EnCa-101 tumors grown in nude mice were removed at 0, 0.5, 1, 2, 4, 6, 12, and 24 hours after subcutaneous E_2 or tamoxifen pellet implantation. Each lane was loaded with 2.0 μg poly(A)$^+$ RNA fractionated in 1.0% agarose gel with 0.66 mol/L formaldehyde transferred to a nylon membrane and hybridized with a v-*fos* probe. *Left panel,* c-*fos* mRNA expression by E_2; *right panel,* c-*fos* mRNA expression by tamoxifen; *bottom panel,* β-actin mRNA expression on same nylon membrane. Membrane hybridized with v-*fos* probe was exposed for 90 hours and with β-actin probe for 2 hours. **B,** Relative expression of protooncogene mRNA in EnCa-101 by E_2 was calculated by densitometric scanning of autoradiographs shown in Figs. 2, *A*, 4, *A*, and 5. Only major 2.7 kb c-*jun* band was scanned and used in the analysis. **C,** Relative expression of protooncogene mRNA in EnCa-101 by tamoxifen was calculated by densitometric scanning of autoradiographs shown in Figs. 2, *A*, 4, *A*, and 5. Data of major 2.7 kb c-*jun* band were used in analysis.

teen mice were subcutaneously implanted with a 1.7 mg E_2 pellet. Four mice received no pellets and served as controls. After E_2 pellet implantation, blood (200 μl) was removed from the retroorbital sinus at indicated time periods. Serum levels of E_2 were determined by radioimmunoassay.

Results

Effect of E_2 and tamoxifen on poorly differentiated EnCa-138 growth. We previously reported on the growth-promoting effects of E_2 and tamoxifen on the E_2-sensitive tumors EnCa-101 and EnCa-X.[2-4] In the present study the growth rate of the poorly differen-

tiated EnCa-138 tumor in nude mice was investigated (Fig. 1). EnCa-138 tumor grew rapidly, and administration of E_2 or tamoxifen did not alter the growth rate. All tumors were devoid of E_2 or progesterone receptors by ligand binding assay (results not presented).

Serum E_2 levels in nude mice subcutaneously implanted with E_2 pellets. In the nude mouse system E_2 and other agents were delivered to the animals continuously by subcutaneous implants of pellets. E_2 pellet was designed to maintain a serum level of 200 to 300 pg/ml over a 60-day period. Thirty minutes after pellet implantation, E_2 levels in serum reached 13 ng/ml and the level was maintained for the next 6 hours. Thereafter E_2 concentration varied between 2.7 and 5 ng/ml for 7 days (Table I).

Induction of c-*fos* mRNA by E_2 and tamoxifen in EnCa-101. To study the effects of E_2 and tamoxifen on immediate, early genes, we examined c-*fos* mRNA expression in E_2- and tamoxifen-sensitive EnCa-101. After E_2 administration a significant induction (Fig. 2, *A* and *B*) was apparent within 1 hour (eightfold) and reached peak level (16-fold) at 2 hours. It was maintained for at least 4 hours, followed by a rapid decrease at 12 hours. C-*fos* mRNA expression was restimulated and reached a high level (13-fold) at 24 hours. Long-term study indicated that c-*fos* expression appeared to by cyclic (not shown). A similar pattern was observed in another steroid receptor-positive, E_2- and tamoxifen-sensitive tumor, EnCa-X, with both E_2 and tamoxifen treatments (not shown). Tamoxifen also induced c-*fos* mRNA expression in EnCa-101 (Figs. 2, *A* and *C*). This induction was more rapid and short-lived than that by E_2. The maximal induction occurred at 30 minutes, and c-*fos* mRNA decreased to below basal level at 6 hours. The peak c-*fos* mRNA level was lower than that obtained with E_2. At 24 hours, c-*fos* mRNA returned to basal level.

Effects of E_2 and tamoxifen on c-*fos* mRNA expression in EnCa-138. To investigate whether c-*fos* mRNA induction was mediated by the steroid hormone pathway, we examined the effects of E_2 and tamoxifen on c-*fos* mRNA expression in the poorly differentiated, steroid receptor-negative, adenosquamous EnCa-138. The steady-state level of c-*fos* mRNA in untreated EnCa-138 tumor was much higher than that in EnCa-101 (Fig. 3). Surprisingly, a small degree of transient induction was observed after E_2 or tamoxifen administration. Within 1 hour of the c-*fos* mRNA level was induced fivefold compared with untreated tumor. It was rapidly decreased to prestimulation levels at 2 hours in E_2-treated tumor and at 4 hours in tamoxifen-treated tumor.

Protooncogene expression in EnCa-101 and EnCa-138. Because c-*fos* and c-*jun* form heterodimers to enhance transcription, we investigated whether c-*jun* mRNA was similarly induced by E_2 and tamoxifen, as was observed with c-*fos*. Hybridization of EnCa-101 and

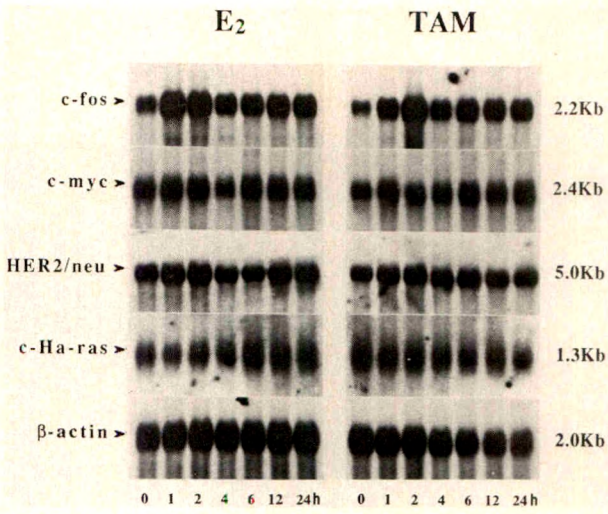


Fig. 3. Northern blot analysis of protooncogene mRNA expression by E_2 and tamoxifen *(TAM)* in EnCa-138. Poorly differentiated EnCa-138 tumors grown in nude mice were removed at 0, 1, 2, 4, 6, 12, and 24 hours after subcutaneous E_2 or tamoxifen pellet implantation. Each lane was loaded with 5.0 μg poly(A) positive RNA. *Left panel,* c-*fos,* c-*myc,* HER-2/*neu,* c-Ha-*ras* and β-actin mRNA expression modulated by E_2; *right panel,* mRNA expression of the same protooncogenes modulated by tamoxifen. Exposure times were as follows: c-*fos,* 20 hours; c-*myc,* 5 hours; HER-2/*neu,* 65 hours; c-Ha-*ras,* 20 hours; and β-actin, 1 hour.

EnCa-138 mRNA with c-*jun* probe showed two bands, a 2.7 kb major species and a 3.3 kb minor species (Fig. 4). Compared with c-*fos* mRNA levels, c-*jun* transcripts were more abundant in untreated tumors of both EnCa-101 and EnCa-138. In EnCa-101, the steady-state levels of c-*jun* mRNA were elevated at 4 hours after E_2 administration (Fig. 4, *A*). In EnCa-138, c-*jun* mRNA appeared to be induced slightly by E_2 and tamoxifen (threefold) (Fig. 4, *B*). The transcripts of other protooncogenes examined either did not show significant changes (c-*myc,* HER-2/*neu,* c-Ha-*ras*) (Fig. 5), were very faint (c-*erb*B not shown), or were undetectable (N-*myc* and c-*fms* not shown).

Comment

The results presented here on the induction of the protooncogene c-*fos* and its expression by E_2 and tamoxifen, provide further support for the E_2-like effects of tamoxifen in human endometrial carcinoma grown in nude mice. We previously reported on the paradoxic, E_2-like effect of tamoxifen on the growth of human endometrial carcinomas EnCa-101 and EnCa-X in the nude mouse model.[3, 5] The c-*fos* mRNA and its protein product, *Fos,* were essentially undetectable in most quiescent cells and attained measurable levels in response to mitogenic stimulation in vitro.[18, 19] Because transcriptional activation of c-*fos* was one of the earliest nuclear events, a role for this oncogene in the cell cycle was suggested.[18, 19] The results of our studies with EnCa-101 in vivo showed that c-*fos* mRNA level

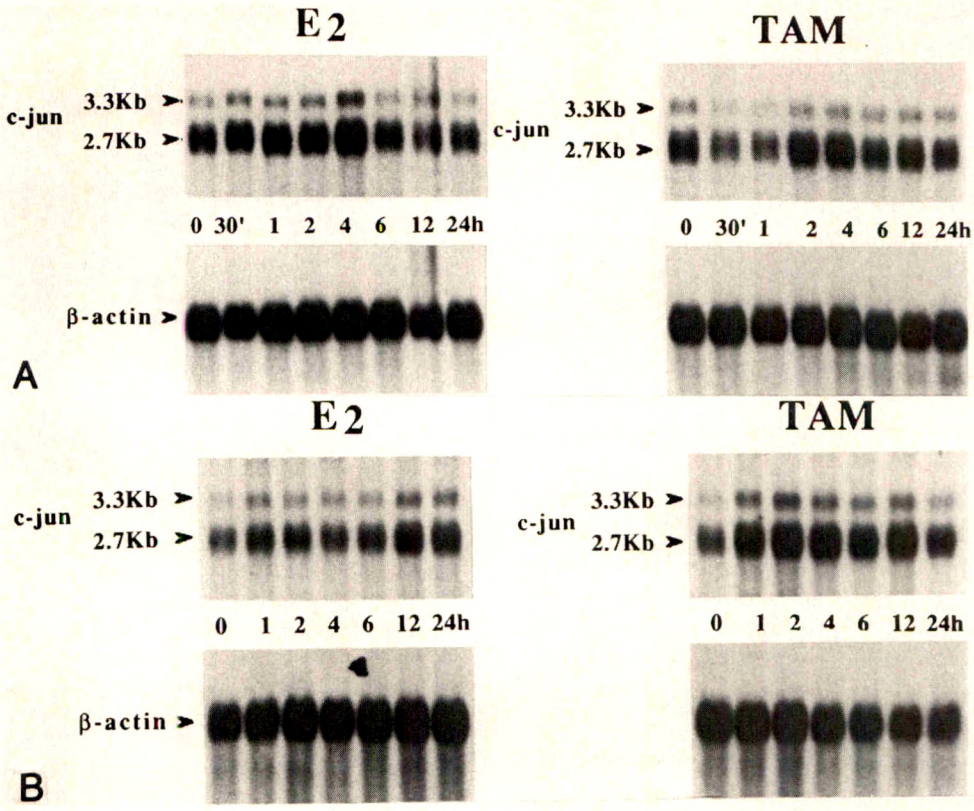


Fig. 4. A, Northern blot analysis of c-*jun* mRNA expression by E_2 and tamoxifen *(TAM)* in EnCa-101. Same RNA samples analyzed in Fig. 2, *A*, were hybridized to a c-*jun* probe. *Left panel* shows c-*jun* mRNA expression modulated by E_2 in EnCa-101; *right panel*, c-*jun* mRNA expression modulated by tamoxifen in EnCa-101 (both autoradiographs were exposed for 20 hours); *Lower panel*, β-actin mRNA expression (exposure time 2 hours). **B,** Northern blot analysis of c-*jun* mRNA expression by E_2 and tamoxifen in EnCa-138. Same RNA samples analyzed in Fig. 3 were hybridized with c-*jun* probe. *Left panel*, c-*jun* mRNA expression modulated by E_2 in EnCa-138 (exposure time 20 hours); *right panel*, c-*jun* mRNA expression by tamoxifen (exposure time 20 hours); *lower panel*, β-actin mRNA expression (exposure time 1 hour).

Table I. Serum E_2 levels after subcutaneous E_2 pellet implantation

Time (hr)	*Serum E_2 levels (ng/ml)*
0	0
0.5	13.3 ± 1.4
1	9.6 ± 1.5
2	11.9 ± 2.9
4	9.7 ± 0.9
6	9.5 ± 1.4
12	4.7 ± 0.3
24	3.0 ± 0.5
36	2.7 ± 0.2
48	2.7 ± 0.3
72	4.3 ± 0.6
96	5.0 ± 0.5
168	3.7 ± 0.7

Male BALB/c mice (n = 22) were castrated 1 week before this study. Eighteen mice were subcutaneously implanted with 1.7 mg E_2 pellets and four mice were left free of implants. Blood (200 μl) from groups (n = 3) of mice was removed from retroorbital sinus at indicated times after E_2 pellet implantation. Serum levels of E_2 were measured by radioimmunoassay in individual samples and expressed as mean ± SEM.

was very low or undetectable in the absence of E_2; its induction became evident within 1 hour, reached peak level at 2 hours, and this level was maintained until 4 hours. Interestingly, the c-*fos* expression pattern induced by tamoxifen was similar to that by E_2, although the peak level attained with tamoxifen (sixfold) was lower than that with E_2 (16-fold). It is noteworthy that under identical growth conditions in our experimental system the growth rate of EnCa-101 induced by E_2 was significantly higher than that observed with tamoxifen.

Although the sequence of events involved in E_2-mediated induction of c-*fos* is unclear, it is possible that E_2–estrogen receptor complex binds to specific estrogen response elements to regulate c-*fos* expression.[20] Tamoxifen–estrogen receptor complex may bind to estrogen response elements in a similar fashion, resulting in modulation of c-*fos* expression. An estrogen response element upstream of human c-*fos* gene that binds estrogen receptor and activator protein 1 transcription factor has been identified.[21] Recently, activation of the

ovalbumin gene by the estrogen receptor was shown to involve the *Fos-Jun* complex.[22] Several recent studies have demonstrated that formation of *Jun-Fos* heterodimers resulted in a remarkable increase in its affinity to activator protein-1 recognition sequence to initiate transcription. In contrast, *Fos* does not appear to form homodimers and is ineffective in binding to specific DNA sequences by itself.[23-25] We observed a relatively higher level of c-*jun* mRNA in EnCa-101 under basal conditions. Exposure to E_2 resulted in a twofold to threefold increase at 4 hours in this system (Fig. 4, *A*). It is conceivable that once c-*fos* protein synthesis occurs in response to E_2, it could instantly form heterodimers with the relatively abundant *Jun* and initiate the sequence of events that mediate the growth-promoting effect of E_2 in EnCa-101. Thus the rapid and transient expression of c-*fos* may be the regulatory step. In contrast to E_2 tamoxifen induced a transient decrease in c-*jun* expression in our study, although significant levels of c-*jun* transcripts were still present at these early time points (Fig. 4, *A*).

EnCa-138 tumor was chosen as a negative control because its growth was unaffected by E_2 and tamoxifen (Fig. 1). Surprisingly, both E_2 and tamoxifen exhibited rapid and transient induction of c-*fos* mRNA in this tumor. However, in contrast to EnCa-101, in which c-*fos* basal level was very low or virtually undetectable, EnCa-138 contained a relatively high level of c-*fos* mRNA in the absence of E_2 or tamoxifen (Fig. 3). The basal level of c-*jun* transcripts was also high and further stimulated twofold to fourfold in the presence of E_2 and tamoxifen (Fig. 4, *B*). A relatively faster growth rate of EnCa-138 compared with EnCa-101 in the nude mouse and the inability of E_2 and tamoxifen to further increase the growth rate of this tumor could explain the higher basal levels of both c-*fos* and c-*jun* mRNA and presumably their protein products.

Recently induction of c-*fos* and c-*jun* mRNA expression by E_2 was reported in the rodent uterus. In addition, expression of other protooncogenes c-*myc*, N-*myc*, c-Ha-*ras* and c-*erb*B was also shown to be modulated by E_2 in rat uterus and human breast carcinoma cell lines.[8-15] We tested eight protooncogenes, c-*fos*, c-*jun*, c-*myc*, N-*myc*, HER2/*neu*, c-*erb*B, c-*fms*, and c-Ha-*ras*. Among them, E_2- and tamoxifen-modulated expression of c-*fos* was several-fold compared with the others examined in the steroid hormone–responsive, well-differentiated EnCa-101.

In summary, we have shown that E_2 transiently enhanced the steady-state levels of c-*fos* mRNA and c-*jun* expression was modulated to a lesser degree by E_2 in human endometrial carcinoma in vivo. The pattern of c-*fos* expression induced by tamoxifen was similar to that observed with E_2. The effect of tamoxifen on immediate early protooncogene expression observed in our study is in accord with its growth-promoting role in human endometrial carcinoma and further distinguishes it as an estrogen agonist in this tissue.

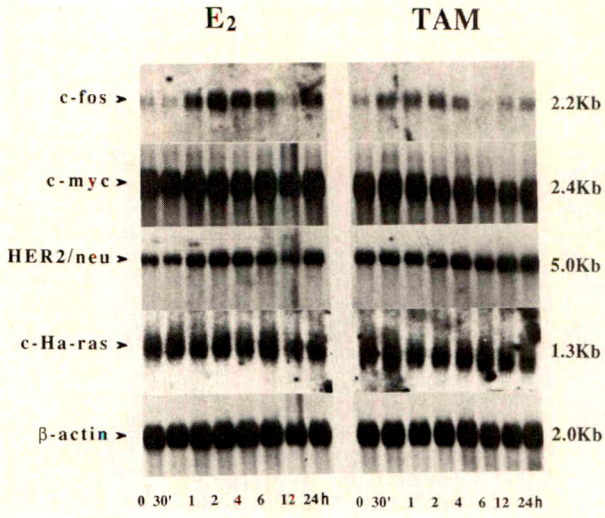


Fig. 5. Northern blot analysis of protooncogenes mRNA expression by E_2 and tamoxifen *(TAM)* in EnCa-101. Same RNA samples analyzed in Fig. 2, *A*, were hybridized to c-*myc*, HER-2/*neu*, and c-Ha-*ras* probes individually. *Left panel* shows c-*fos*, c-*myc*, HER-2/*neu*, c-Ha-*ras*, and β-actin mRNA expression modulated by E_2; *right panel* shows same protooncogene mRNA expression modulated by tamoxifen. Exposure times for autoradiographs were as follows: c-*fos*, 90 hours; c-*myc*, 20 hours; HER-2/*neu*, 20 hours; c-Ha-*ras*, 65 hours; and β-actin, 2 hours.

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Low-dose infusion of atrial natriuretic peptide in the conscious guinea pig increases blood flow to the placenta of growth-retarded fetuses

Thomas B. Jansson, MD, PhD
Göteborg, Sweden

OBJECTIVE: Our goal was to investigate the effects of low-dose infusion of atrial natriuretic peptide on maternal placental blood flow of normal-sized and growth-retarded fetuses.
STUDY DESIGN: Seventeen conscious, chronically catheterized guinea pigs were studied at day 50 of gestation after growth retardation was experimentally induced by unilateral uterine artery ligation at day 34.
RESULTS: In 12 experiments atrial natriuretic peptide infusion (15 ng/kg/min) increased blood flow (microsphere technique) to placentas of growth-retarded fetuses by 26% ($p < 0.01$) while placental blood flow of normal-sized fetuses remained unchanged. This difference in responsiveness to atrial natriuretic peptide infusion was statistically significant ($p < 0.001$). Blood pressure, heart rate, and myoendometrial and renal blood flows were unaffected. Plasma concentrations of immunoreactive atrial natriuretic peptide increased by 64%, indicating that physiologic concentrations were achieved. In five saline solution–treated controls none of the studied parameters changed.
CONCLUSION: Atrial natriuretic peptide in low doses selectively increases blood flow to placentas of growth-retarded fetuses in the conscious guinea pig. Infusion of atrial natriuretic peptide in a dose that results in plasma concentrations within the upper physiologic range may have a therapeutic value in pregnancies complicated by intrauterine growth retardation. (Am J Obstet Gynecol 1992;166:213-8.)

Key words: Intrauterine growth retardation, placental blood flow, atrial natriuretic peptide, placenta, uteroplacental vasculature

Except for estrogens,[1] few substances have been shown to dilate the maternal vessels supplying the placenta in late pregnancy. Although it may be argued that the placental vascular bed is maximally dilated near term, it was recently demonstrated that stimulation of adenylate cyclase by forskolin dilates the vessels of the near-term ovine placenta.[2]

Systemic administration of a potent vasodilator in pharmacologic doses decreases blood pressure and inevitably elicits increased levels of circulating catecholamines. Because uteroplacental vessels are highly sensitive to the vasoconstricting effects of norepinephrine,[3,4] the possible vasodilating effects of the substance will be counteracted, making interpretation of results difficult. This problem can to some extent be circumvented by local administration. However, when such potent endogenous vasodilators as prostacyclin[5] and adenosine[6] were infused close to the uterine artery in the sheep, no placental vasodilation could be demonstrated.

Atrial natriuretic peptide (ANP) is an endogenous substance with diuretic, natriuretic, and hypotensive effects.[7] ANP stimulates insoluble guanylate cyclase, thereby increasing the intracellular levels of cyclic guanosine monophosphate. Circulating levels of ANP have been reported to increase in pregnancy compared with nonpregnant controls,[8] although others have been unable to demonstrate differences.[9] Because ANP is released mainly from the right atrium and acts as a circulating hormone, it is appropriate to administer this peptide systemically to study its physiologic effects. Interestingly, ANP given in high doses dilates the maternal placental vasculature in the late pregnant anesthetized rat[10] and in the near-term sheep.[11] In contrast, in a recent report uterine vascular resistance was unaffected by ANP infusion in the late pregnant sheep.[12]

Pharmacologic doses of ANP elicit cardiovascular effects that are markedly different compared with the effects induced by doses of ANP that result in concentrations within the physiologic range (low dose).[7] The objective of this investigation was therefore to study the effects of low-dose infusions of ANP on placental blood flow in the chronically catheterized conscious guinea pig. An additional purpose was to study whether the

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vessels supplying the placenta of growth-retarded fetuses differ in vascular reactivity to ANP.

Material and methods

The investigations described in this paper were approved by the local ethical committee for animal experimentation (170/88).

Seventeen nulliparous albino guinea pig does of the Dunkin-Hartley strain were used. The animals were allowed free access to food (standard pellets and hay) and water (with ascorbic acid, vitamin E, and selenium added). The animals were maintained in an environment of controlled humidity (40% to 70%) and temperature (18° to 20° C), with a 12-hour light-darkness cycle. The day of mating (day 0) was determined by daily inspection of the vaginal membrane. Initially two females and one male guinea pig were housed together; after pregnancy was confirmed by palpation of the fetuses at days 15 to 20 of gestation the does were transferred to a special room in the animal facilities and kept in separate cages. From day 20 until the day of experimentation animals were fed 0.2 to 0.3 ml of water daily from a syringe to accustom them to handling.

At 31 to 38 (34.3 ± 0.4, mean ± SEM) days' gestation the animals were anesthetized with 3% to 4% enflurane and nitrous oxide/oxygen (1 : 1). Under aseptic conditions and temperature control (rectal temperature 37.5° to 38.5° C) a 2 to 3 cm lower abdominal midline incision was made. The mesometrial fat pad of one of the uterine horns was exposed and the uterine artery ligated. The operation lasted 10 to 15 minutes, and the animals were standing up in their cages within 10 minutes of completion.

At 41 to 46 (44.3 ± 0.4) days' gestation, animals were anesthetized with ketamine hydrochloride (30 mg subcutaneously) and xylazine (3 mg intramuscularly), preceded by atropine (0.05 mg subcutaneously). Polyethylene catheters (Becton Dickinson, Parsippany, N.J.) were inserted into the femoral artery and advanced to the aortic bifurcation (PE 25, inner diameter 0.40 mm, connected to PE 90, inner diameter 0.86 mm), into the left ventricle by cannulation of the right common carotid artery in the retrograde direction (PE 50, inner diameter 0.58, connected to PE 90), and into the right external jugular vein (PE 50, connected to PE 90). The catheters were tunneled subcutaneously and exteriorized between the scapulae. A small amount of antibiotic paste (Socatyl vet, Ciba-Geigy, Basel, Switzerland) was applied in the wounds before closure. The operation lasted 30 to 60 minutes. During and after the operation the animals were given a total volume of 30 ml of physiologic saline solution intravenously over 2½ hours. Isoprenaline was added to the fluid for an infusion rate of 1.5 μg/kg/min to preserve cardiac output during anesthesia. Glucose was given (10 mg/kg/min) in the last 20 ml. Catheters were filled with heparinized saline solution and sealed. The animals recovered within 3 to 5 hours after the operation. To maintain patency the femoral artery catheter was flushed with heparinized saline solution every 24 hours.

At least 5 days after the catheterization (49 to 52 days' gestation) experiments were performed in the animal room with the animal freely moving in its own cage without any visual contact with the investigator. After the animal was connected to a swivel it was allowed 2 hours to accommodate to the experimental situation. Throughout the experiment arterial blood pressure and heart rate were measured by means of a pressure transducer (Statham Gould P23 ID, Hato Rey, Puerto Rico) and an oscillation detector, respectively. All recordings were made on a polygraph (No. 7, Grass Instrument, Quincy, Mass.).

Placental blood flow was measured by means of the microsphere technique. Microspheres with a diameter (mean ± SD) of 16.5 ± 0.1 μm and labeled with either niobium 95, ruthenium 103, or cerium 141 (Du Pont, D-6072 Dreieich, Germany) were used. The microspheres were suspended in saline solution with Tween 80. After vigorous vibration with a whirl mixer, 0.6 to 0.7 ml (corresponding to 400,000 spheres) was injected into the left ventricle catheter for 45 seconds; the catheter was then flushed with 1 ml saline solution for 45 seconds. A reference blood sample was withdrawn from the abdominal aorta at a rate of 0.65 ml/min from 10 seconds before the start of the microsphere injection until 75 seconds after the end of flushing. Donor blood was given to compensate for blood loss.

Fifteen minutes after the first microsphere injection, which represents basal blood flows, an intravenous infusion of ANP (15 ng/kg/min, 0.06 ml/min) was started in 12 experiments. ANP (rat sequence, Sigma Chemical Co., St. Louis) was dissolved in physiologic saline solution with bovine serum albumin (0.5 gm/100 ml) added. In five experiments only saline solution with bovine serum albumin was given. Thirty minutes after the start of infusion a second microsphere injection was carried out. In some experiments in the ANP group, blood was obtained from the abdominal aorta immediately after the two microsphere injections for analysis of plasma catecholamine (0.6 ml, n = 10) and ANP concentrations (2 ml, n = 5). Blood samples were promptly transferred to prechilled ethyleneglycol-bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid-glutathione– (catecholamines) or ethylenediaminetetraacetic acid–containing (ANP) tubes on ice and subsequently centrifuged (+4° C). Plasma was stored at −50° C until analysis. Blood loss was compensated for with donor blood.

The animal was killed with an overdose of barbiturate. All catheter positions were checked and found

adequate. The uterus was cut between the vagina and cervix and divided through a midline incision of the cervix into the two horns. The uterine horns were opened and the placentas detached from the uterine wall through the separation zone between the subplacenta and the main placenta. Individual placentas and myoendometrium and kidneys were weighed, cut into smaller pieces, and placed into counting vials. The radioactivity of the samples was determined in a multichannel-pulse height analyzer (Packard 5019 connected to Packard 3320, Packard Instrument Co., Inc., Downers Grove, Ill.). Organ blood flows were calculated by the following formula: Organ blood flow (ml/min) = (No. of microspheres in organ/No. of microspheres in reference blood sample) × withdrawal rate (ml/min). No experiment contained fewer than 720 microspheres in placentas, kidneys, or reference blood sample. The lower blood flow (milliliters per minute per gram) of the two kidneys was divided by the higher blood flow value, giving a ratio ≤1.0 for each microsphere injection; the mean ratio was 0.95 ± 0.01 (range 0.83 to 1.0), indicating an adequate mixing of spheres. A specific radioimmunoassay method that included human antibody and guinea pig serum was used to analyze immunoreactive ANP; in other respects the technique was identical to the method described in detail previously.[13] Plasma catecholamine concentrations were analyzed by high-performance liquid chromatography with electrochemical detection.[14]

Results are expressed as mean ± SEM. In cases of more than one normal-sized or growth-retarded fetus in a horn, a mean value for the placental blood flow was calculated for that horn. Student's t test for paired or unpaired observations was used for statistical analysis. To evaluate differences in vascular reactivity between groups, relative changes of blood flows from basal values were used.

Results

Only experiments with at least one growth-retarded fetus in the ligated horn were included in the study; growth retardation was arbitrarily defined as >15% weight reduction compared with the mean fetal weight in the control horn. Maternal weights and gestational ages are given in Table I. In the growth-retarded group of the ANP series (n = 12) fetal weight was reduced by 45%, placental weight by 38%, and placental blood flow (ml/min/gm) by 58%. Corresponding reductions were seen in the control group (n = 5, Table II).

ANP infusion did not affect blood pressure, heart rate, or renal or myoendometrial blood flow (Table III). ANP administration (15 ng/kg/min) increased maternal plasma concentrations of immunoreactive ANP by 64%; plasma catecholamine concentrations did not change (Table IV). ANP infusion increased maternal placental blood flow of growth-retarded fetuses by 26% ($p < 0.01$), and blood flow to normal-sized placentas was unaltered (Fig. 1). This difference in reactivity to ANP between supplying vessels of growth-retarded and normal-sized placentas was statistically significant ($p < 0.001$). The correlation between these paired relative changes in blood flow in response to ANP was highly significant (r = 884, $p < 0.001$, n = 12); this correlation provides a statistical justification for using the paired t test. Central hemodynamics and organ blood flows remained unchanged in the control group.

Table I. General characteristics of ANP and control groups at day of experiment

	ANP group (mean ± SEM)	*Control group (mean ± SEM)*
n	12	5
Gestational age (days)	50.5 ± 0.3	49.8 ± 0.5
Maternal weight (gm)	907 ± 33	842 ± 37

Comment

Whether plasma concentrations of ANP are increased in pregnancy compared with nonpregnant controls is still a matter of controversy.[8, 9] In addition, if ANP levels are elevated in normal pregnancy the physiologic role of this increase remains unclear. In nonpregnant experimental animals ANP decreases cardiac output and plasma volume and antagonizes the renin-angiotensin system[7] in contrast to the hypervolemia, increased cardiac output, and stimulation of the renin-angiotensin system seen in normal pregnancy. Because the main stimulus for ANP release is increased tension in the right atrial wall caused by intravascular volume expansion,[7] ANP release might be enhanced because of the hypervolemic state of normal pregnancy.

In this study infusion of ANP did not affect blood flow to normal-sized placentas in contrast to findings in the anesthetized rat[10] and the chronically catheterized sheep.[11] Although these differences may be caused by interspecies variation, an alternative explanation is the use of low doses of ANP (15 ng/kg/min) in the present investigation. This should be compared with the dose of 30 μg/kg given systemically in the study of Chemtob et al.[10] and 6.25 μg/min infused locally in the uterine artery in the investigation of Reid et al.[11] Basal ANP plasma concentrations in the present study were within the range reported for arterial concentrations in laboratory animals.[7] In the conscious dog[15] plasma ANP concentrations may increase twofold to fivefold in response to physiologic stimuli such as volume loading. Thus it is highly likely that the 64% increase of the

Table II. Fetoplacental weights and basal placental blood flows

	ANP group		*Control group*	
	Normal-sized (mean ± SEM)	*Growth-retarded (mean ± SEM)*	*Normal-sized (mean ± SEM)*	*Growth-retarded (mean ± SEM)*
Fetal weight (gm)	40.4 ± 1.6	22.4 ± 1.8†	41.7 ± 3.0	28.9 ± 3.3†
Placental weight (gm)	4.00 ± 0.23	2.47 ± 0.20†	4.62 ± 0.66	3.27 ± 0.43*
Placental blood flow (ml/min/gm)	2.34 ± 0.23	0.99 ± 0.11†	2.54 ± 0.28	1.04 ± 0.09*

*$p < 0.01$ compared with normal-sized, paired t test.
†$p < 0.001$.

Table III. Central hemodynamics and renal and myoendometrial blood flows

	ANP group		*Control group*	
	Basal (mean ± SEM)	*Infusion (mean ± SEM)*	*Basal (mean ± SEM)*	*Infusion (mean ± SEM)*
Blood pressure, (mm Hg)	58.5 ± 2.0	59.3 ± 2.4	58.6 ± 1.4	60.2 ± 0.9
Heart rate (beats/min)	269 ± 8	274 ± 7	289 ± 4	293 ± 8
Renal blood flow (ml/min/gm)	4.81 ± 0.41	4.40 ± 0.34	3.39 ± 0.28	3.56 ± 0.16
Myoendometrial blood flow (ml/min/gm)				
Nonligated horn	0.16 ± 0.02	0.17 ± 0.02	0.19 ± 0.05	0.17 ± 0.04
Ligated horn	0.19 ± 0.01	0.21 ± 0.02	0.20 ± 0.03	0.16 ± 0.04

None of the changes were statistically significant. ANP was infused at the rate of 15 ng/kg/min. Controls were given saline solution.

Table IV. Maternal plasma ANP and catecholamine concentrations

	n	*Basal (mean ± SEM)*	*ANP infusion (mean ± SEM)*
ANP (pg/ml)	5	145.4 ± 6.2	239.2 ± 13*
Norepinephrine (pmol/ml)	10	2.61 ± 0.36	3.51 ± 0.55
Epinephrine (pmol/ml)	10	0.56 ± 0.12	0.70 ± 0.05

*$p < 0.001$.

levels of circulating ANP during ANP infusion represent concentrations within the physiologic range.

The relevance of the uterine artery ligation model in the study of placental blood flow regulation in intrauterine growth retardation has recently been addressed in some detail.[16] Fetal growth in the ligated horn is not only affected by an impairment in placental blood flow caused by the experimental manipulation but may also be influenced by, for example, the number of fetuses in the litter in general and the number of fetuses in the horn in particular. Although mean fetal weight in late gestation and mean birth weight is inversely proportional to litter size in the guinea pig, this effect cannot clearly be demonstrated before day 55 of gestation.[17] Thus fetal load characteristics are unlikely to interfere significantly in this investigation. It is also possible to estimate the effect of increasing the number of fetuses in one horn by comparing, in litters with a total of three fetuses, mean fetal weight in a control horn containing two fetuses with the mean fetal weight in a control horn with one fetus. When this was carried out with data from the present series of experiments and two similar studies from our laboratory,[16, 19] an increase in the number of fetuses from one to two in a horn decreased fetal weight by 4.5%. Thus the effect of the number of fetuses in the horn on fetal growth is likely to be only modest. In addition the mean number of fetuses in the present investigation was identical in the ligated and control horns.

To support the relevance of this experimental model of intrauterine growth retardation, there is evidence that the placenta of a small fetus produced by uterine artery ligation is qualitatively similar to the placenta of a fetus being small because of crowding effects or a small fetus occurring spontaneously as a runt. For example, the placenta that is small because of uterine artery ligation is characterized by the same degree of blood-flow reduction,[4, 18, 19] exhibits identical placental glucose and amino acid transfer characteristics,[18] and shows similar reactivity to norepinephrine[4] and prostaglandin synthesis inhibition[19] as does the placenta of the naturally occurring runt.

The striking difference in response to ANP infusion between normal-sized and growth-retarded placentas may be caused by an active up-regulation of ANP receptors in vessels supplying the small placenta. ANP receptors have been identified in non-brush border microsomal fractions from the guinea pig[20] and human

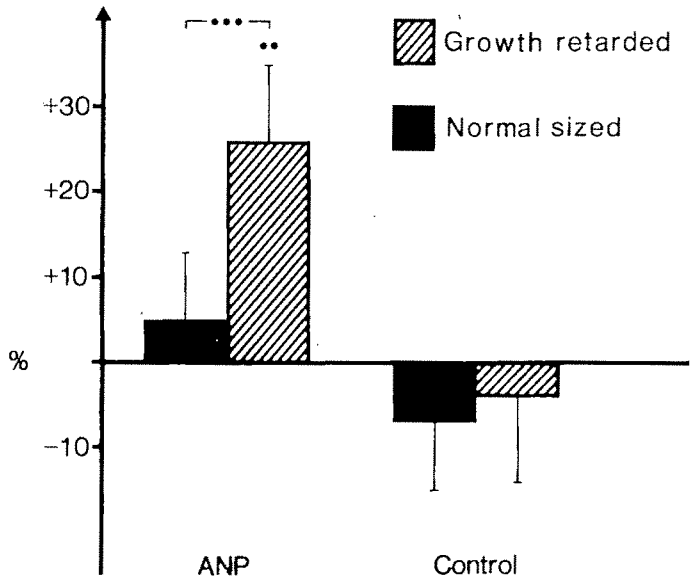


Fig. 1. Effect of ANP (15 ng/kg/min, $n = 12$) and saline infusions (control, $n = 5$) on blood flow to placentas of normal-sized and growth-retarded fetuses. *Two asterisks,* $p < 0.01$: difference between basal blood flow and blood flow during ANP infusion in the growth-retarded group; *three asterisks,* $p < 0.001$; difference in relative blood flow changes between normal-sized and growth-retarded placentas.

placenta.[21] To my knowledge no investigation has been undertaken to directly identify the presence of ANP receptors in the uteroplacental vasculature.

An alternative explanation to the current findings may be that the vessels supplying the normal-sized guinea pig placenta are maximally dilated at day 50 of gestation (0.74 gestation). The demonstration of vasodilatory capacity of the near-term ovine placental vascular bed (0.90 gestation) by local infusion of forskolin[2] suggests that this is less likely. However, infusions of forskolin or higher doses of ANP in the guinea pig would provide direct evidence.

A third possibility is that physiologic doses of ANP dilate placental vessels earlier in gestation but that this ability progressively disappears during the course of pregnancy. The responsiveness of vessels supplying the growth-retarded placenta to ANP at day 50 could be explained by a delayed development or maturation of the growth-retarded placenta and its vascular supply. Such delay has been described for different aspects of placental development in intrauterine growth retardation. For example, normal physiologic changes in uteroplacental vessels during pregnancy such as the gradual disappearance of normal muscular and elastic tissue in the vessel wall have been shown to be inadequate in idiopathic growth retardation.[22] Consequently it is possible that the vessels supplying the growth-retarded placenta contain relatively more smooth muscle compared with the placenta of normal-sized fetuses. This may be the basis for the higher ANP sensitivity of the growth-retarded placental vasculature in these experiments. In addition, blood vessels supplying the growth-retarded placenta are substantially less sensitive to circulating catecholamines as compared with the vasculature of normal-sized placenta.[19] It was suggested that this difference may be caused by a delayed sympathetic denervation to these vessels. If this holds true, the selective vasodilatory effect of ANP could result from a higher density of remaining sympathetic vasoconstrictor nerves around the growth-retarded placenta because ANP inhibits norepinephrine release from nerve endings in isolated preparations.[23] The higher sensitivity of the normal-sized placentas to circulating catecholamines[19] could explain the differential effects of ANP infusions on placental blood flow. However, the finding that plasma catecholamine concentrations were unaffected by ANP infusion precludes this possibility.

The finding that low-dose infusion of ANP markedly dilates the vessels of the growth-retarded placenta suggests that physiologic doses of the peptide may improve placental blood flow and thereby fetal outcome in intrauterine growth retardation. In the study of Chemtob et al.,[10] in which pharmacologic doses of ANP decreased blood pressure and increased placental blood flow in the anesthetized hypertensive rat, the authors suggested that ANP may have therapeutic potential in hypertensive pregnancies. However, chronic administration of high doses of ANP is likely to substantially reduce plasma volume, which is already contracted in pregnancy-induced hypertension. As a consequence, the acute beneficial effects of ANP administration may

be counteracted. With infusions of ANP in doses resulting in plasma concentrations within the physiologic range, such side effects are likely to be much less pronounced. Speculatively, a moderate volume load may be an alternative approach to increasing placental blood flow in pregnancies with intrauterine growth retardation. However, this requires the ability to respond to volume loads with an increased ANP secretion to be conserved in pregnancy. This seems to be the case in several animal species, for example, the rat.[24] In the human being, however, a volume load increased ANP levels in pregnancies complicated by pregnancy-induced hypertension[25] but not in normal pregnancies.[9] Administration of ANP in addition to a volume load may be an additional possibility. Because hemodilution per se preferentially increases blood flow to placentas of small fetuses without reducing oxygen flow in the guinea pig,[26] a volume load may also be beneficial for rheologic reasons.

In conclusion, ANP infused in physiologic doses improves blood flow to placentas of growth-retarded fetuses in the conscious guinea pig without affecting central hemodynamics, plasma catecholamines, or renal or myoendometrial perfusion. The vascular supply of placentas of normal-sized fetuses showed a different reactivity in that blood flow remained unaltered to these placentas in response to ANP. It is speculated that a low-dose infusion of ANP may have a therapeutic value in pregnancies complicated by intrauterine growth retardation.

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The effect of preovulatory peritoneal fluid from cases of endometriosis on murine in vitro fertilization, embryo development, oviduct transport, and implantation

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OBJECTIVE: The null hypothesis of our study is that the success of in vitro and in vivo murine fertilization and embryo development is not decreased by gamete exposure to peritoneal fluid from superovulated patients with endometriosis.
STUDY DESIGN: A murine in vitro fertilization model was used to test the effects of endometriosis versus nonendometriosis peritoneal fluid at concentrations of 1%, 5%, and 10% versus an unsupplemented control. Fertilization and blastocyst formation were compared by analysis of variance. In a second experiment superovulated mice were given intraperitoneal injections of endometriosis or nonendometriosis fluid or saline solution 8 hours after human chorionic gonadotropin and then mated. Some mice were killed 3 days after coitus to assess embryo number, cleavage stage, and uterine versus tubal position by means of analysis of variance and covariance with repeated measures. Others were killed 12 days after coitus with the mean number of implantations per animal between groups compared by Student's *t* test.
RESULTS: In vitro fertilization rates decreased as peritoneal fluid concentration increased in both the endometriosis (65%, 43%, 33%) and nonendometriosis (65%, 52%, 35%) groups at 1%, 5%, and 10% peritoneal fluid concentration, respectively. Mice receiving intraperitoneal endometriosis or nonendometriosis fluid or saline solution injections showed no differences in embryo number, cleavage, uterine versus tubal position, or mean implantation number.
CONCLUSION: Peritoneal fluid from superovulated patients had no differentially negative effect when compared with the effect of nonendometriosis peritoneal fluid on murine in vitro or in vivo fertilization and embryo development, tubal embryo transport, or implantation. (Am J Obstet Gynecol 1992;166:219-24.)

Key words: Endometriosis, in vitro fertilization, murine model

The relationship between endometriosis and infertility remains to be clarified. The literature contains conflicting reports with regard to whether peritoneal fluid prostaglandin (PG) levels are elevated in patients with endometriosis.[1-4] Studies have pointed to greater peritoneal macrophage levels and in vitro sperm phagocytosis activity in endometriosis patients as a possible explanation of how endometriosis could decrease fertility.[5-9] However, other studies, including one we reported, failed to verify increased phagocytic activity.[10, 11] One recent study has reported that culture media from peritoneal fluid macrophages obtained from endometriosis patients significantly inhibited sperm penetration of zona-free hamster eggs.[12] In support of these findings, another study reported peritoneal fluid from endometriosis patients inhibited murine in vitro fertilization.[13] A problem with that study, however, was the lack of control in cycle time when peritoneal fluid specimens were collected. The large differences in cellular and hormonal composition of peritoneal fluid at different times during the menstrual cycle make this an important consideration.[11, 12]

The purpose of our study was to investigate further the effect of preovulatory endometriosis peritoneal fluid from superovulated women on mouse in vitro fertilization and subsequent embryo development. In addition, we used the murine model to evaluate the in vivo effect of endometriosis peritoneal fluid on early embryo development, tubal embryo transport, and implantation.

Material and methods

Subjects. Peritoneal fluid was collected from the anterior and posterior cul-de-sac at the time of laparoscopic oocyte retrieval in patients undergoing in vitro fertilization (IVF) or gamete intrafallopian tube transfer (GIFT) procedures. All patients were superovulated with the same human menopausal gonadotropin–follicle-stimulating hormone controlled ovarian hyperstimulation protocol. Peritoneal fluid was collected

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from IVF and GIFT patients so that all specimens would be preovulatory. Blood-contaminated specimens were discarded. The peritoneal fluid was immediately filtered with a 0.22 μm filter (Millipore, Bedford, Mass.) and then snap-frozen in liquid nitrogen vapor to −140° C. It was then subsequently stored at −80° C until used. Each subject had a complete evaluation of the peritoneal cavity for the presence and extent of endometriosis. Ten consecutive endometriosis and 10 nonendometriosis specimens were collected for study. All patients underwent staging according to the American Fertility Society revised classification system.[14] Five of the endometriosis patients had prior laparoscopic diagnosis of endometriosis. There had been no medical or surgical treatment for endometriosis in any of these five patients for >6 months before this study. The other five patients were newly diagnosed as having endometriosis at the time of IVF or GIFT laparoscopy. The nonendometriosis control peritoneal fluid specimens were obtained from the tubal and male factor infertility patients undergoing IVF and GIFT, respectively. All of the women in both the endometriosis and nonendometriosis groups had negative indirect serum Immunobead sperm antibody studies. All specimens were coded by number to allow blinding of the laboratory technicians performing the various assays. A total of 10 endometriosis and 10 nonendometriosis peritoneal fluid specimens were analyzed for each assay described in the subsequent sections.

Mouse in vitro fertilization assay. Juvenile CB_6F_1 female mice and sexually mature CB_6F_1 male mice (Charles River, Wilmington, Mass.) were the sources of the oocytes and sperm. This strain has consistently provided in vitro fertilization results of 70%. Ovulation was induced with intraperitoneal pregnant mare serum gonadotropin followed 48 hours later by 7.5 IU human chorionic gonadotropin (hCG) also given intraperitoneally. The females were killed by cervical dislocation 13 to 16 hours after hCG. The oviduct was excised and placed in 2.5 ml of Whitten's fertilization media containing 30 mg/ml crystalline bovine serum albumin. A sterile needle was used to incise the oviduct and liberate the oocyte clumps. The oocyte clumps were then pooled and placed into 0.5 ml drops of Whitten's fertilization media and overlaid with mineral oil. Males were killed by cervical dislocation 1 hour before females were killed. Both cauda epididymides were removed and placed into 0.9 ml Whitten's fertilization media. Sperm was extracted with jeweler's forceps by applying gentle pressure on the cauda epididymis. The sperm suspension was examined at 10 minutes for the presence of whirlpool formation, and a concentration was determined with a Makler chamber. Ninety minutes after sperm collection, the oocytes were inseminated with 100 μl of sperm suspension by means of a micropipette. Fertilization media were supplemented in a blinded fashion with peritoneal fluid from nonendometriosis and endometriosis patients. An unsupplemented Whitten's media group served as another control group. Peritoneal fluid concentrations of 1%, 5%, and 10% were evaluated. Endometriosis and nonendometriosis peritoneal fluid and Whitten's control specimens were run in parallel fashion for each concentration with a minimum of 100 oocytes evaluated for each test specimen. After 6 hours in Whitten's fertilization media, all oocytes were transferred to Whitten's growth media containing 3 mg/ml bovine serum albumin. Oocytes from the endometriosis and nonendometriosis 1%, 5%, and 10% supplemented fertilization media were transferred to appropriately matched 1%, 5%, or 10% endometriosis or nonendometriosis peritoneal fluid–supplemented Whitten's growth media. Fertilization and embryo culture were carried out in a modular incubator chamber (Billups-Rothenberg, Del Mar, Calif.) in a trigas mixture of 90% nitrous oxide, 5.5% oxygen, 4.5% carbon dioxide. Fertilization defined at the two-cell cleavage stage was assessed at 24 hours. Two-cell embryos from test and control groups were then transferred to fresh, unsupplemented Whitten's growth media. Embryos were cultured for 96 hours after insemination and blastocyst transformation rate was scored under phase contrast microscopy.

In vivo mouse assay. CD-1 female mice (Charles River) underwent ovulation induction in a fashion similar to that performed in the in vitro fertilization mouse assay with 7.5 IU pregnant mare serum gonadotropin given intraperitoneally followed by 7.5 IU hCG intraperitoneally 48 hours later. Eight hours after hCG, which represented approximately 2 to 3 hours before ovulation, the female mice were given an intraperitoneal injection of 0.2 ml of peritoneal fluid from either the endometriosis or nonendometriosis study specimens. Six mice were tested with each patient's peritoneal fluid specimen. A control group of six mice given an intraperitoneal injection of 0.2 ml of normal saline solution also underwent follow-up. Each female was placed in an individual mating cage with a CD-1 male. Vaginal plug formation was evaluated at 24 hours. A minimum of three successfully mated animals for each peritoneal fluid tested and each control group was required for evaluation. At 72 hours the females were killed by cervical dislocation. The fallopian tubes and uterus were flushed separately, and the number of embryos and stage of development for both tube and uterus were scored.

In vivo mouse implantation assay. Ovulation induction and mating of CD-1 female and male mice was carried out as in the preceding in vivo mouse assay section. Again, 0.2 ml intraperitoneal injections of endometriosis and nonendometriosis peritoneal fluid were tested along with a 0.2 ml intraperitoneal normal saline solution control group. Again intraperitoneal in-

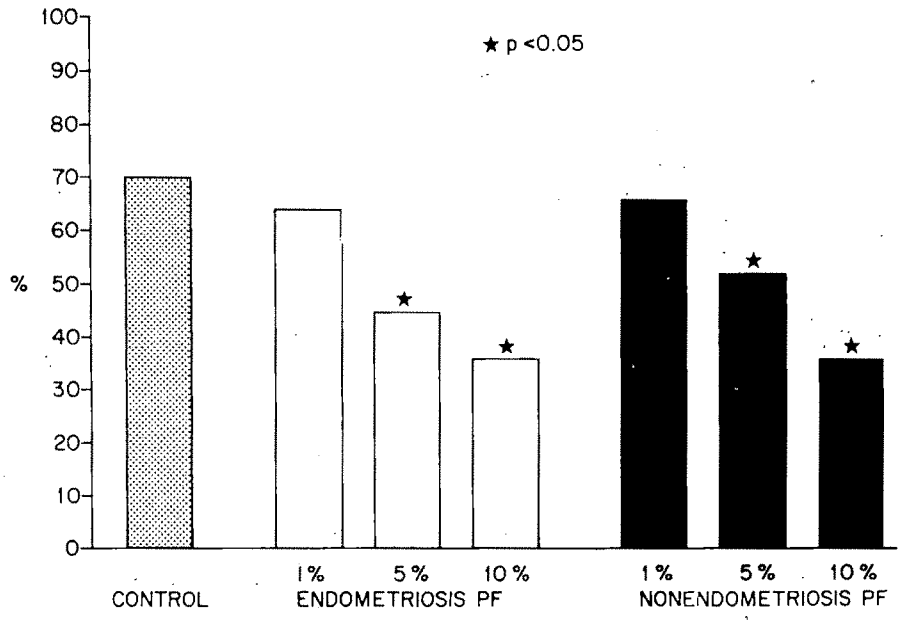


Fig. 1. Percent of oocytes fertilized in vitro is shown for nonsupplemented control and peritoneal fluid–supplemented endometriosis and nonendometriosis groups. There was no significant difference in oocyte fertilization between control, 1% endometriosis, and 1% nonendometriosis groups. There was a significant decrease in oocyte fertilization in both endometriosis and nonendometriosis peritoneal fluid groups as percent of supplemented peritoneal fluid was increased.

jections were given 8 hours after hCG. Ten animals for each patient specimen were tested in parallel runs. A minimum of four successfully mated animals in each test and control group was required for evaluation. Females were killed 12 days after coitus and number of embryos was scored for each group.

Data analysis. In vitro fertilization and blastocyst transformation rates were analyzed by analysis of variance. Evaluation of in vivo embryo development and tubal versus uterine position between groups was by analysis of variance and covariance with repeated measures. Mean number of gestations per animal between groups in the in vivo mouse implantation assay was compared by Student *t* test.

Results

A comparison of the mean patient age (32 ± 5.2 vs 33 ± 5.7 years) and time of hCG and estradiol administration (1442 ± 432 vs 1518 ± 506 days) between the endometriosis and nonendometriosis groups revealed no significant differences. The endometriosis patients consisted of two patients with stage II, six with stage III, and two with stage IV disease. All but one had endometriosis involving at least one ovary.

As depicted in Fig. 1, there was a progressive decrease in the in vitro fertilization rates as the peritoneal fluid concentration increased in both the endometriosis and nonendometriosis peritoneal fluid groups. There was no difference, however, in fertilization rates between the two groups when the rates were compared at similar concentrations of peritoneal fluid. In addition, there was no impairment of subsequent two-cell embryo development in the peritoneal fluid groups compared with the unsupplemented media control group. Blastocyst transformation and embryo degeneration rates were equal among the three groups.

Table I summarizes the results of embryo development and tubal transport from the second group of experiments in which periovulatory mice were given an intraperitoneal injection of endometriosis or nonendometriosis peritoneal fluid or saline solution. The mean number of blastocysts, morula, and two- to eight-cell, one-cell, and degenerated embryos and their distribution in the oviducts and uterus at 3 days after coitus were similar in all three groups. The mating and conception rates (not depicted in Table I) were also similar in all three groups. The 12-day postcoitus implantation results of those mice given periovulatory intraperitoneal injections of endometriosis, nonendometriosis versus saline solution control are summarized in Table II. Again, there was no difference in the mean number of gestational sacs among the groups.

Comment

The role of endometriosis in causing infertility is clear when there is significant adhesive disease involving the fallopian tubes or when there is extensive destruction of the ovaries by endometrioma. However, in patients with mild or moderate endometriosis without pelvic adhesive disease the relationship to infertility is

Table I. In vivo tubal-uterine embryo transport

	Blastocyst	*Morula*	*Two- to eight-cell*	*One-cell*	*Degenerated*
Oviduct					
Endometriosis peritoneal fluid	0.9 ± 1.3	4.6 ± 5.3	1.4 ± 2.1	0.5 ± 1.1	0.7 ± 1.5
Nonendometriosis peritoneal fluid	1.1 ± 1.9	4.4 ± 4.7	1.8 ± 3.6	0.7 ± 1.4	0.5 ± 1.0
Control	1.0 ± 1.4	4.3 ± 3.6	1.3 ± 3.1	0.4 ± 1.2	0.8 ± 1.2
Uterus					
Endometriosis peritoneal fluid	0.6 ± 1.2	1.9 ± 3.6	0.8 ± 1.5	0.2 ± 0.7	0.1 ± 0.3
Nonendometriosis peritoneal fluid	0.7 ± 1.2	1.7 ± 2.7	1.0 ± 2.2	0.2 ± 0.4	0.1 ± 0.2
Control	0.8 ± 1.3	2.1 ± 2.6	1.2 ± 1.8	0.4 ± 0.5	0.2 ± 0.1

Values are mean ± SD.

Table II. Implantation and embryo development 12 days after coitus

Endometriosis peritoneal fluid	15.7 ± 7.1
Nonendometriosis peritoneal fluid	16.7 ± 7.2
Control	16.4 ± 6.8

Mean ± SD.

unclear. Most recent investigations of endometriosis have focused on local peritoneal factors including peritoneal fluid prostaglandin and leukotriene levels, activity and concentration of peritoneal fluid macrophages, and peritoneal fluid macrophage secretory products. At present, no consensus exists as to whether peritoneal fluid prostaglandins or leukotriene levels are increased in patients with endometriosis because the published studies have yielded conflicting results.[1, 3, 15] A role for pelvic macrophages as potential mediators of infertility in patients with endometriosis has also been studied extensively with equally conflicting results reported.[8-12]

Other researchers have focused on local immunologic changes in patients with endometriosis as a potential mechanism that interferes with fertility. Interleukin-1 and interleukin-2, which are secretory products of activated macrophages and activated T cells, respectively, have been the most thoroughly studied. A study by Hill et al.[16] found that only very high concentrations of interleukin-1 inhibited the in vitro development of mouse embryos from the two-cell to blastocyst stage.[16] In a more recent investigation, Schneider et al.[17] reported that purified recombinant interleukin-1 and interleukin-2 had no effect on in vitro mouse embryo development from the four-cell to blastocyst stage or on early stages of implantation as measured in vitro by attachment and outgrowth of blastocysts to fibronectin-coated dishes.

Schneider et al. also cocultured mouse embryos with activated peritoneal macrophages from patients with endometriosis and found no effect on embryogenesis. These results concurred with our prior finding of no difference in interleukin-1 levels between infertile patients with and without endometriosis.[10] However, it remained possible that interleukin-1, interleukin-2, another cytokine, or an as yet unidentified labile soluble factor in peritoneal fluid could affect ovulation, fertilization, or tubal embryo transport and implantation. In the current study we attempted to address that issue by evaluating periovulatory peritoneal fluid from superovulated infertile endometriosis patients. We felt it important to snap-freeze the peritoneal fluid to −140° C and then store it at −80° C until it was used in our studies in an effort to preserve potentially important labile factors. Our finding of a decrease of in vitro fertilization rates in mice as the concentrations of peritoneal fluid supplemented to the fertilization media were increased was similar to that reported by Sueldo et al.[13] One explanation for the decrease in fertilization rate as the peritoneal fluid concentration increased may be that it is from superovulated patients. As noted in Fig. 1, this decrease was equal in both the endometriosis and nonendometriosis groups. In contradistinction to Sueldo et al., we found no difference in murine in vitro fertilization rates between the endometriosis and nonendometriosis peritoneal fluid groups. Our results are in agreement with the study findings of Halme et al.,[18] who found that peritoneal fluid from endometriosis patients compared with peritoneal fluid from nonendometriosis patients did not adversely affect spermatozoa penetration of zona-free hamster eggs. One criticism of the current study is that if peritoneal fluid from superovulated patients is gamete toxic, then it is possible that subtle adverse effects of endometriosis may not have been detected.

We further cultured the in vitro fertilized embryo to see if there was any subtle effect on the oocyte or early embryo that may be manifested as a decrease in its blastocyst transformation potential. No difference was found between the endometriosis peritoneal fluid, nonendometriosis peritoneal fluid, and unsupplemented control groups. Gametes were only exposed for the first

24 hours of culture of peritoneal fluid supplement to allow us to focus on effects of endometriosis peritoneal fluid on fertilization. We have previously shown that endometriosis peritoneal fluid did not affect in vitro two-cell to blastocyst transformation.[10]

A recent study by Hahn et al.[19] in a rabbit model in which endometriosis was surgically induced found results similar to our in vitro murine endometriosis peritoneal fluid model with respect to the effect of endometriosis on ovulation, fertilization, and early embryo development. In their experiment animals were mated and killed 1, 4, 8, or 14 days after insemination. They flushed the uterus and tubes from animals killed on both day 1 and day 4 after insemination and found results similar to ours with no difference in the number of corpora lutea or fertilized ova between animals with endometriosis and the nonendometriosis controls. However, in those rabbits killed on days 8 and 14, they found a significant decrease in the number of implantation sites. Hahn et al. also collected peritoneal fluid from rabbits with endometriosis and injected it intraperitoneally to a group of normal female rabbits 24 hours before insemination. They reported that this group also had a significant decrease in the number of successful implantations. One possible explanation for this is that the mouse has a bursa connecting its distal tube and ovary together. It may be that this bursa effectively blocks important factors in the intraperitoneally injected endometriosis peritoneal fluid. It could also be a weakness in our in vivo model study design in giving the intraperitoneal fluid only 3 hours before anticipated ovulation. However, the reason we chose to give our intraperitoneal fluid in this manner was to obtain a high concentration of the peritoneal fluid at the time of ovulation to primarily test its effect on fertilization. In testing the pregnant mare serum gonadotropin–stimulated CD-1 female mouse, we had found the periovulatory peritoneal fluid volume to be 0.1 to 0.2 ml; therefore a 0.2 ml intraperitoneal fluid injection represented a substantial test dose.

The lack of an in vivo effect of endometriosis peritoneal fluid in the mouse certainly does not necessarily equate with the effect endometriosis may have in the human. However, in a recent review of endometriosis and human IVF, Oehninger et al.[20] reported that regardless of stage of endometriosis there was no difference in rates of fertilization, embryo cleavage, or pregnancy per embryo transfer between endometriosis and nonendometriosis patients. However, that study did report a higher miscarriage rate and lower ongoing pregnancy rate in those patients with stage III or IV disease. Although that is a retrospective review, it suggests that in more advanced endometriosis cases an as yet undefined factor(s) may adversely affect either embryo quality or the uterine environment. If there are oocyte- or embryo-inhibiting factor(s) produced in patients with advanced endometriosis, their suppressive effects may be manifested more in patients undergoing GIFT because fertilization and early development are in vivo. Further clinical study of both IVF and GIFT success in patients with endometriosis is needed.

In conclusion, this study demonstrated that peritoneal fluid from superovulated endometriosis patients had no differentially negative effect on mouse in vitro fertilization, embryo development, tubal embryo transport, or implantation. Potential subtle effects of endometriosis on embryo development and implantation will require further investigation.

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Attenuation of the vasoconstrictor effects of thromboxane and endothelin by nitric oxide in the human fetal-placental circulation

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OBJECTIVE: We hypothesized that the endothelial-derived relaxing factor nitric oxide may contribute to low resting vascular tone and may attenuate vasoconstrictor action in the human fetal-placental circulation.
STUDY DESIGN: Isolated human placental cotyledons were dually perfused in vitro, and the effects of N-monomethyl-L-arginine and N-nitro-L-arginine (3×10^{-4} mol/L), which are nonmetabolizable analogs of L-arginine, the substrate for nitric oxide synthase, on resting perfusion pressure and on the fetal-placental circulation preconstricted with U46619 (10^{-8} mol/L) or endothelin-1 (10^{-8} mol/L) were established. Responses before and after inhibition were compared by paired *t* test. The effects of glyceryl trinitrate (10^{-6} mol/L), acetylcholine (10^{-4} mol/L), the calcium ionophore A23187 (10^{-6} mol/L), and histamine (10^{-8} to 10^{-4} mol/L) were also determined in the preconstricted fetal-placental circulation.
RESULTS: Both N-monomethyl-L-arginine and N-nitro-L-arginine (3×10^{-4} mol/L) increased resting perfusion pressure ($p < 0.06$), and N-nitro-L-arginine promptly and significantly increased perfusion pressure in the fetal-placental circulation preconstricted with U46619 ($p < 0.0004$) or endothelin-1 ($p < 0.06$). Nitric oxide generated by addition of glyceryl trinitrate (10^{-6} mol/L) attenuated the vasoconstrictor effects of U46619 ($p < 0.026$) or endothelin-1 ($p < 0.01$). Neither acetylcholine nor the calcium ionophore A23187 had an effect on the fetal-placental circulation, whereas bradykinin further increased perfusion pressure. Histamine only relaxed the preconstricted preparations at concentrations (10^{-6} to 10^{-4} mol/L) above those shown to release nitric oxide in other systems.
CONCLUSION: The stimulus to nitric oxide generation in the fetal-placental circulation may be hydrodynamic. Nitric oxide appears to contribute to maintenance of basal vascular tone and to attenuate the actions of vasoconstrictors in this circulation. (Am J Obstet Gynecol 1992;166:224-30.)

Key words: Endothelin, nitric oxide, thromboxane, placenta

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Currently a great wealth of literature is being generated concerning the production and mechanism of action of endothelial-derived vasoactive agents that may act in an autocrine or paracrine manner to regulate vascular tone. These agents include endothelial-derived relaxing factors[1] such as nitric oxide[2] and endothelial-derived constricting factor or endothelin.[3] In the absence of nerves in the umbilical and placental vessels, blood flow in the human fetal-placental circulation

must be regulated by humoral agents or autocrine-paracrine mechanisms. We have previously presented indirect evidence[4] that the vasodilator nitric oxide may be produced by the human fetal-placental circulation in vitro and may contribute to maintenance of basal vascular tone. We further demonstrated that agents which generated nitric oxide in vitro were able to vasodilate the human fetal-placental circulation preconstricted with the thromboxane mimetic U46619. Nitric oxide is synthesized by a calcium, calmodulin, and nicotinamide adenine dinucleotide phosphate–requiring enzyme, nitric oxide synthetase,[5] which has been found in endothelial cells[6] and at several neural locations[7] and which forms nitric oxide from the guanidino nitrogen of the amino acid L-arginine. This discovery has led to the synthesis of several competitive inhibitors of nitric oxide formation,[8] which can be used to provide more specific data on the synthesis and action of nitric oxide. The human fetal-placental circulation can be constricted in a concentration-dependent manner by both thromboxane mimetics[9] and by endothelin-1,[10] a peptide that produces long-acting vasoconstriction. Nitric oxide may be released by the fetal-placental circulation to attenuate the actions of vasoconstrictors in a manner similar to that we have previously described for the vasodilator prostacyclin.[9] We have used two inhibitors of nitric oxide synthesis to further define the action of nitric oxide in the human fetal-placental circulation, particularly the attenuation of vasoconstrictor action, and we have also begun to investigate the mechanisms that stimulate release of nitric oxide in the fetal-placental circulation in vitro.

Material and methods

Placentas were collected immediately after normal vaginal delivery or elective cesarean section and transported to the perfusion laboratory in the labor and delivery area. In this and previous studies,[4, 9, 11] we found no difference in vascular reactivity between tissues obtained at cesarean section and those taken at vaginal delivery. A suitable third- or fourth-order chorionic artery and corresponding vein of an intact cotyledon were cannulated as previously described[11] at a point immediately before passage of the vessels through the chorionic plate. The maternal intervillous space was cannulated with two butterfly needles inserted through remnants of the spiral arteries in the basal plate. The effluent from the intervillous space was collected by gravity into a Plexiglas cone, over which the cotyledon was placed, maternal surface downward. Perfusion medium, except where stated, was tissue culture medium 199 containing 5% polyvinylpyrrolidone 40 and 0.1% bovine serum albumin as oncotic agents and with 20 IU/ml heparin and 48 μg/ml gentamicin added. Medium was pH 7.4 with bicarbonate and gassed with 95% oxygen–5% carbon dioxide at 37° C. Flow rates were 4 ml/min for fetal tissue and 10 ml/min for maternal tissue except where stated. Lateral pressure was measured in fetal and maternal inflow lines adjacent to the point of cannulation. Data on pressure and inflow and outflow P_{O_2} were sampled every second and stored on an IBM PS2 system with Asystant Plus software (Asyst Technologies, Rochester, N.Y.).

The effect of agonists that stimulate endothelium-derived relaxing factor formation in other vascular systems was investigated. The fetal-placental circulation was preconstricted by addition of the thromboxane mimetic U46619 (10^{-8} mol/L) to the fetal perfusate and by constant perfusion with this solution. When the fetal-placental circulation had constricted and perfusion pressure had equilibrated, a 2 ml bolus of acetylcholine (10^{-4} mol/L), the calcium ionophore A23187 (10^{-6} mol/L), or bradykinin (10^{-6} mol/L) was infused into the fetal-placental circulation and changes in perfusion pressure were recorded. Similar experiments were performed with infusion of boluses of increasing concentrations of histamine (10^{-8} to 10^{-4} mol/L) into the preconstricted fetal-placental circulation and recording of changes in perfusion pressure.

The effects of two competitive inhibitors of nitric oxide synthetase were next investigated. The perfusion medium normally used (medium 199) contains 3×10^{-4} mol/L L-arginine, the substrate for nitric oxide synthesis; therefore the medium for these experiments was changed to Hanks' buffered saline solution, pH 7.4, containing oncotic agents, heparin, and antibiotics as before and gassed with 95% oxygen–5% carbon dioxide. After establishment of the perfusion, a 30-minute period of equilibration was allowed for washout of endogenous L-arginine before addition of either N^G-monomethyl-L-arginine (NMLA, 3×10^{-4} mol/L) or N^G-nitro-L-arginine (NNLA, 10^{-3} mol/L) to both the maternal and fetal perfusates. Changes in perfusion pressure were recorded throughout. In a separate series of experiments, U46619 (10^{-8} mol/L, constant infusion) or endothelin-1 (10^{-8} mol/L, 2 ml bolus infusion) was added to the fetal perfusate after the washout period to constrict the fetal-placental circulation. When perfusion pressure had reequilibrated at the increased level, NNLA (10^{-3} mol/L) was added to both maternal and fetal perfusates, and perfusion was continued with changes in perfusion pressure being recorded.

All experiments were repeated in at least four separate placentas. U46619 was kept as a 10^{-3} mol/L stock in ethanol at −20° C and diluted before use. Endothelin-1 was stored at 10^{-5} mol/L in 0.5 mol/L acetic acid at −70° C. NMLA and NNLA were made fresh as 10^{-3} mol/L solutions before use. The vasodilator response to histamine was expressed as percent relaxation from

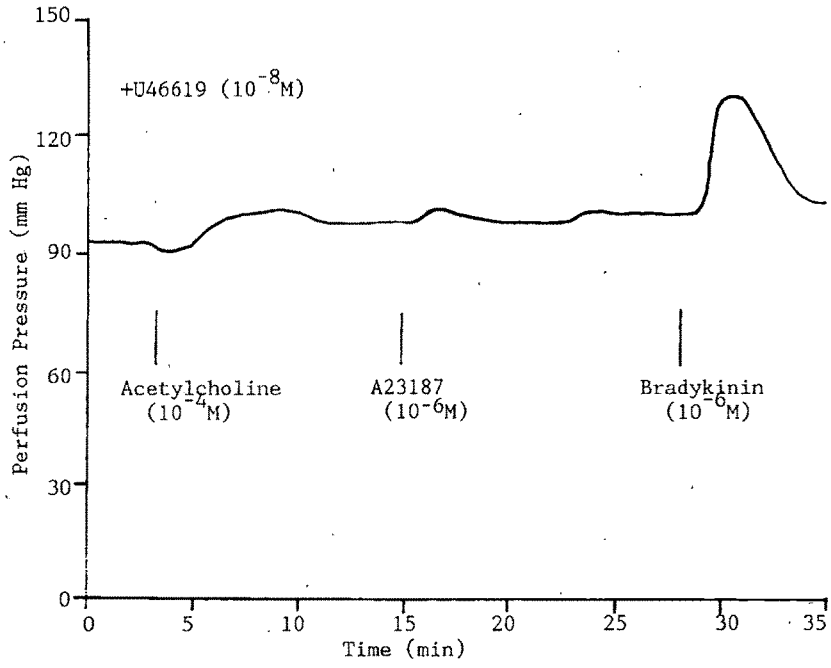


Fig. 1. Representative experiment of effect of acetylcholine (10^{-4} mol/L), ionophore A23187 (10^{-6} mol/L), or bradykinin (10^{-6} mol/L) on perfusion pressure in fetal-placental circulation preconstricted with U46619 (10^{-8} mol/L).

the resting perfusion pressure in each placenta. Vasoconstrictor responses to NMLA and NNLA were expressed as percent change from resting perfusion pressure in each placenta. Changes in perfusion pressure were analyzed by analysis of variance or by paired *t* test where appropriate. All procedures were approved by the University of Cincinnati College of Medicine Institutional Review Board.

Results

Acetylcholine (10^{-4} mol/L) and the calcium ionophore A23187 (10^{-6} mol/L) had no significant effect on perfusion pressure (increases in pressure of 1.3 ± 0.4 and 5.1 ± 0.9 mm Hg, respectively) in the preconstricted human fetal-placental circulation whereas bradykinin (10^{-6} mol/L) consistently gave further vasoconstriction (mean ± SEM 19.4 ± 8.1 mm Hg, $n = 5$) in addition to that already caused by the U46619 (Fig. 1). However, histamine was able to significantly attenuate ($p < 0.0001$, analysis of variance) U46619-induced vasoconstriction in a concentration-dependent manner from 10^{-6} to 10^{-4} mol/L (Fig. 2).

No difference in resting perfusion pressure was seen regardless of whether medium 199 or Hanks' buffered saline solution was used as perfusion medium. Addition of either NMLA (3×10^{-4} mol/L) or the more potent inhibitor of nitric oxide synthesis,[12] NNLA (10^{-3} mol/L), to perfusion medium increased resting perfusion pressure; however, the increase did not reach statistical significance ($p < 0.06$, Fig. 3). When the fetal-placental circulation was preconstricted with U46619 (10^{-8} mol/L), addition of NNLA (10^{-3} mol/L) produced a prompt further significant increase ($p < 0.027$) in perfusion pressure (Fig. 4, Table I). The increase in perfusion pressure reached a maximum after 15 minutes and then started to decline and returned with time to a pressure not significantly different from that before addition of NNLA (Fig. 4). The difference in perfusion pressure between the maximum obtained on addition of NNLA and that on return to baseline was significant ($p < 0.027$, data not shown).

Endothelin-1 (10^{-8} mol/L) given as a bolus significantly increased perfusion pressure ($p < 0.004$, Table I). Addition of NNLA (10^{-3} mol/L) to perfusion medium then increased perfusion pressure further ($p < 0.06$). In separate experiments glyceryl trinitrate (10^{-6} mol/L) significantly reduced ($p < 0.01$) perfusion pressure in the fetal-placental circulation preconstricted with endothelin-1 (Table II) in agreement with the reduction in perfusion pressure by glyceryl trinitrate ($p < 0.026$) in the fetal-placental circulation preconstricted with U46619.

Comment

We have previously demonstrated[4] that agents which generate nitric oxide (glyceryl trinitrate and S-nitroso-

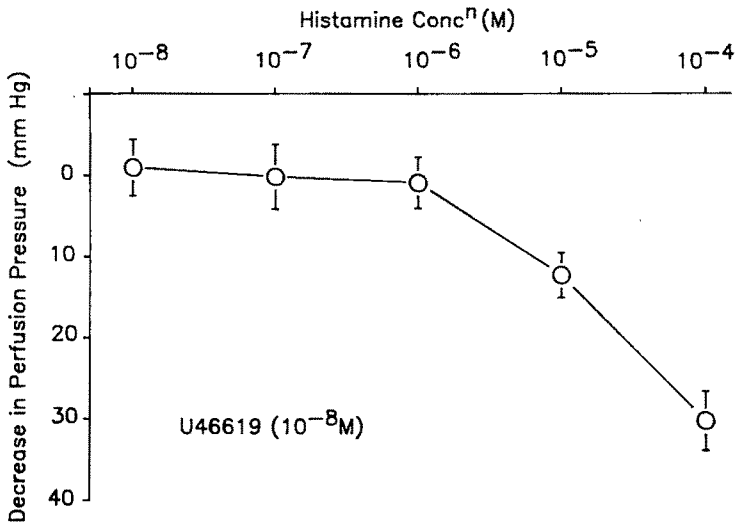


Fig. 2. Effect of histamine on preconstricted fetal-placental cotyledon. Fetal-placental vasculature of isolated cotyledons was preconstricted with continuous infusion of U46619 (10^{-8} mol/L) and then increasing concentrations of histamine (10^{-8} to 10^{-4} mol/L) were infused as a 2 ml bolus. Change in perfusion pressure was expressed as percent decrease from resting pressure (mean ± SEM, $n = 5$ experiments). One-way analysis of variance showed significant decrease in perfusion pressure with increasing concentration of histamine ($p < 0.0001$). Decrease in perfusion pressure at 10^{-5} and 10^{-4} mol/L histamine was significantly different from that at 10^{-6} mol/L histamine ($p < 0.05$, Student–Newman-Keuls).

N-acetyl-penicillamine) were able to relax the human fetal-placental vasculature preconstricted in vitro with a thromboxane mimetic. By using methylene blue, an inhibitor of endogenous nitric oxide action, we further provided evidence for a role of nitric oxide in maintenance of basal vascular tone in this preparation and demonstrated that inhibition of the action of nitric oxide on guanylate cyclase gave small but significant increases in resting perfusion pressure. Using the derivatives of L-arginine (NNLA and NMLA), which are competitive inhibitors of nitric oxide synthetase, we are currently able to demonstrate further increases in perfusion pressure in the preconstricted fetal-placental circulation by addition of these compounds and presumptive inhibition of nitric oxide formation. Although addition of these inhibitors to perfusion medium did increase resting perfusion pressure, the increase did not quite reach significance ($p < 0.06$). Interestingly, this contrasts with our previous finding[4] that methylene blue, which inhibits the action of nitric oxide, significantly increased perfusion pressure, but this lack of significant effect may be due to the continuing presence of endogenous L-arginine in the preparation, which is forming nitric oxide and competing with the inhibitor in spite of the use of Hanks' buffered saline solution as perfusate. The concentration of L-arginine in placental tissues is 3×10^{-4} mol/L, so it is possible that complete depletion of endogenous substrate for nitric oxide synthetase cannot be easily achieved by perfusion with Hanks' buffered saline solution. Gude et al.[15] did report that addition of NNLA, methylene blue, or oxyhemoglobin was able to significantly increase resting perfusion pressure when Krebs solution was used as perfusate. The reasons for the disparity with our findings, other than the different experimental technique, are not readily apparent from their report.

The effect of the competitive inhibitor NNLA in the preconstricted circulation was rapid in onset, reached a plateau after about 15 minutes, but then apparently was lost as perfusion pressure returned to the level seen before addition of the analog. In other in vitro systems[14] the effect of such competitive inhibitors is purportedly only reversible on addition of L-arginine, the substrate for nitric oxide synthetase. A primary function of the placenta is the active transport of amino acids from mother to fetus, and the placenta contains a high concentration of L-arginine; therefore this endogenous pool may be competing with the inhibitor as we have described. To minimize this effect, we replaced our normal perfusion medium (medium 199), which itself contains L-arginine, with Hanks' buffered saline solution and preperfused the preparation for 30 minutes to wash out amino acids. However, the lack of persistent increase in perfusion pressure on addition of the inhibitor suggests either that there is still either sufficient endogenous L-arginine present, that release of another

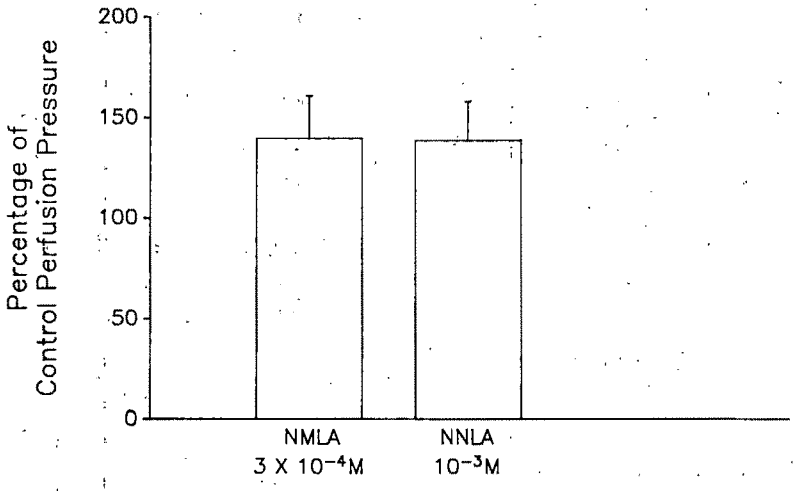


Fig. 3. Effect of competitive inhibitors of nitric oxide synthesis on resting perfusion pressure in fetal-placental circulation. Effect of bolus infusion of either NMLA (3×10^{-4} mol/L) or NNLA (10^{-3} mol/L) on resting perfusion pressure in fetal-placental vasculature. Resulting perfusion pressure was expressed as percent of resting pressure (mean ± SEM, $n = 5$ experiments).

Table I. Effect of inhibition of nitric oxide synthetase on U46619 and endothelin-induced vasoconstriction

		Perfusion pressure (mm Hg)		
	No.	*Control*	*With agonist*	*With agonist plus NNLA (10^{-3} mol/L)*
U46619 (10^{-8} mol/L)	5	22.6 ± 2.6	119.7 ± 18.1*	175.5 ± 22.9†
Endothelin-1 (10^{-8} mol/L)	4	44.7 ± 10.5	81.9 ± 11.6‡	94.4 ± 10.7§

Values are mean ± SEM. Significance determined by paired t test.
*Significant increase from control, $p < 0.0022$.
†Significant difference from agonist alone, $p < 0.027$.
‡Significant increase from control, $p < 0.004$.
§Significant difference from agonist alone, $p < 0.062$.

compensatory vasodilator occurs as pressure increases, or that protein breakdown may be occurring in the placenta perfused with Hanks' buffered saline solution to release amino acids (L-arginine) that could be converted to nitric oxide.

In an attempt to establish the stimulus to release of nitric oxide in the perfused cotyledon, we studied the effect of vasodilator agonists that are normally thought to act in other systems via release of nitric oxide. Neither acetylcholine nor the calcium ionophore A23187 was able to vasodilate the preconstricted cotyledon. The absence of effect of acetylcholine on the perfused cotyledon and on the release of endothelium-derived relaxing factor from umbilical vessels has been previously reported,[15,16] and Griggs and McLaughlin[17] showed that A23187 could not relax preconstricted human chorionic plate arteries. In agreement with other reports,[18] we found that bradykinin was a vasoconstrictor in this preparation and indeed further increased perfusion pressure. To demonstrate vasodilation, we needed higher concentrations of histamine (10^{-5} to 10^{-4} mol/L) than those shown to be effective in other systems,[19] including release of endothelium-derived relaxing factor from umbilical vein.[16] However, this finding is in contrast to that of Griggs and McLaughlin,[17] who showed 10^{-6} mol/L histamine to further constrict preconstricted chorionic plate arteries. The stimulus to nitric oxide release in the fetal-placental vasculature is therefore more likely to be pressure or flow-shear stress in the vascular bed. Our previous report[4] has demonstrated an apparent flow-induced release of nitric oxide.

Inhibition of nitric oxide formation by addition of nonmetabolizable analogs of L-arginine, although having no significant effect in our hands on resting perfusion pressure, further increased the vasoconstriction induced by both U46619 and endothelin-1. Although the effect on endothelin-1–induced vasoconstriction failed to reach significance ($p < 0.06$), nitric oxide generated by addition of glyceryl trinitrate was able to sig-

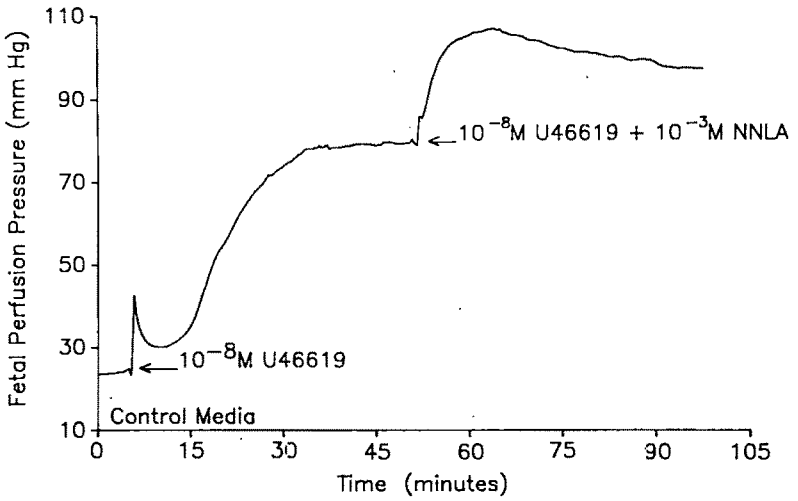


Fig. 4. Effect of N^G-nitro-L-arginine on perfusion in preconstricted fetal-placental vasculature. Representative tracing is shown of effect of infusion of NNLA (10^{-3} mol/L) on perfusion pressure in fetal-placental vasculature preconstricted by constant infusion of U46619 (10^{-8} mol/L).

Table II. Effect of generation of nitric oxide on U46619 and endothelin-induced vasoconstriction

	No.	*Perfusion pressure (mm Hg)*		
		Control	*With agonist*	*With glyceryl trinitrate (10^{-5} mol/L)*
U46619 (10^{-8} mol/L)	4	22.6 ± 2.6	116.5 ± 14.0*	77.6 ± 9.1†
Endothelin-1 (10^{-8} mol/L)	4	25.8 ± 2.6	77.9 ± 9.2‡	59.0 ± 8.7§

Values are mean ± SEM. Significance determined by paired *t* test.
*Significant increase from control, $p < 0.0002$.
†Significant difference from agonist alone, $p < 0.026$.
‡Significant increase from control, $p < 0.01$.
§Significant difference from agonist alone, $p < 0.01$.

nificantly relax the fetal-placental circulation preconstricted with endothelin-1, in agreement with our previous findings against U46619-induced vasoconstriction.[4] Therefore nitric oxide does appear to be formed to act as a compensatory vasodilator to attenuate the actions of both vasoconstrictors. Interestingly, there appears to be a heterogeneity of sensitivity of responsiveness to endothelin-1 between human arteries and veins, with greater sensitivity in veins and within the venous system.[20] In arteries, nitric oxide inhibited endothelin-1–induced vasoconstriction but was less effective in veins, although it appeared to be effective against norepinephrine-induced vasoconstricted veins. The relative production and effectiveness of nitric oxide between arteries and veins are as yet unknown.

In summary, by using competitive inhibitors, we have obtained evidence that the formation of nitric oxide contributes to the maintenance of resting vascular tone and attenuates the action of vasoconstrictor agents in the human fetal-placental circulation. The stimulus to formation of this nitric oxide may be physical or hydrodynamic or may be a specific stimulus by the vasoconstrictor agent itself. However, it does occur in response to vasoconstriction by both the thromboxane mimetic U46619 and by endothelin-1.

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Influence of terbutaline on ovine uterine response to prostaglandin E_2 challenge

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OBJECTIVE: Our purpose was to test the effects of terbutaline on uterine electric and contractile responses to prostaglandin E_2.

STUDY DESIGN: In five late-gestation ewes, prostaglandin E_2 (22.9 ± 2.3 μg/min for 3 minutes) was given twice at 30-minute intervals during control. Terbutaline sulfate (2 μg/min) was then infused for 30 minutes. Prostaglandin E_2 challenge was repeated 10 minutes after the onset of terbutaline infusion and thereafter at 30-minute intervals. Two-way analysis of variance for repeated measures revealed a significant main effect for time ($p \leq 0.0001$) and between time and response ($p \leq 0.05$).

RESULTS: Both electric ($p \leq 0.0001$) and intrauterine pressure ($p \leq 0.0001$) responses were suppressed during terbutaline. The influence on intrauterine pressure persisted 10 minutes after terbutaline ($p \leq 0.01$) while the electric response was not different from control.

CONCLUSIONS: Terbutaline initially diminishes both uterine contractile and electric activity, but electric recovery precedes contractile recovery. (Am J Obstet Gynecol 1992;166:231-5.)

Key words: Tocolysis, terbutaline, sheep, excitation-contraction coupling

Terbutaline, a short-acting β_2 sympathomimetic, is a commonly used agent for prevention of preterm labor.[1-3] The tocolytic power of β_2-adrenergic-receptor agonists is mediated by activation of adenyl cyclase and increases in myometrial intracellular cyclic adenosine monophosphate. The rise in cyclic adenosine monophosphate is responsible for decreased intracellular concentration of calcium and decreased activity of myosin light-chain kinase at a given calcium concentration.[4, 5, 6] The β_1-and β_2-adrenergic-receptor agonist isoprenaline has been reported to weaken the coupling between contraction and excitation in smooth muscles.[7] We have reported a similar action produced by systemic infusion of forskolin, a compound that also stimulates increased intracellular cyclic adenosine monophosphate.[8]

We designed an experiment to determine the effects of terbutaline tocolysis on the electric and contractile activities of the pregnant ovine myometrium. The uterine electromyogram and intrauterine pressure responses to repeated prostaglandin E_2 challenges were measured to determine the time course of the action of terbutaline infusion on myometrial excitation and contraction in response to prostaglandin E_2 challenge.

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Material and methods

All guidelines for the care and use of animals as approved by the University of Wisconsin were followed throughout the course of these experiments. Operations were performed on five time-bred pregnant ewes on day 125 ± 1 of gestation. Food and water were withheld from each ewe for 24 hours before surgery. Ketamine (10 mg/kg) and atropine (10 μg/kg) were administered intramuscularly to induce anesthesia and to reduce tracheal secretions, respectively. An anesthetized state was maintained throughout surgery by intravenous ketamine that was infused through a jugular vein catheter and supplemented when necessary by pentobarbital sodium. Surgical protocol followed our standard procedure.[9] A fetal hind limb was accessed through a midline laparotomy and hysterotomy. Vascular catheters were inserted into an artery and vein, and the tips were advanced to positions in the abdominal aorta and inferior vena cava, respectively. A third catheter with side holes was anchored to the incision site to monitor intrauterine pressure. The uterine incision was then repaired. Three Cooner (Cooner Sales Co., Chatsworth, Calif.) electrode wires were tied into the myometrium to acquire the uterine electromyogram signal. Catheters were inserted bilaterally into the maternal hind limb arteries, and the tips were advanced 20 to 30 cm. Catheters and electrode leads were exteriorized through the maternal flank and stored in a cloth pouch; the laparotomy was then repaired. A catheter was placed in a maternal carotid artery and advanced; placement in the left ventricle was confirmed at surgery when a left ventricular waveform was ap-

parent on an R711 dynograph chart recorder (Beckman Instruments, Inc., Fullerton, Calif.). The maternal jugular vein and left ventricular catheters were wrapped in gauze and secured to the neck with a loosely wrapped elastic bandage. Antibiotics were administered to the fetus (chloramphenicol, 100 mg/kg), intrauterine space (penicillin, 600,000 U), and ewe (penicillin, 400,000 U administered intramuscularly). Each ewe was then placed in a clean pen and given free access to food and water for the 5-day recovery period.

Experimental protocol. On the day before the experiment, each ewe was moved to the laboratory area and housed in a mobile pen. All experiments were performed at approximately the same time of day. On the morning of the experiment a 1 ml maternal arterial sample was removed for determination of blood gas tensions, pH, and hematocrit on a pH and blood gas analyzer (System 1302, Instrumentation Laboratory, Inc., Lexington, Mass.) and a Select-A-Fuge 24 centrifuge (Bio-Dynamics, Indianapolis). Measurements were made at 37° C and converted by algorithm to appropriate values at 39° C. Pressure signals were calibrated on the PIDAS data acquisition system (PIDAS, Ithaca, N.Y.) and pressure transducers (models P23KL and P23Db, Gould, Inc., Oxnard, Calif.) with use of a mercury manometer. The uterine electrodes were connected to a junction box that fed signals to the PIDAS unit through a low-pass filter set at 50 Hz. Analog signals were digitized on line and averaged every 5 seconds. Both analog and digital signals were recorded on magnetic tape. Analog signals were also recorded on a chart recorder (model 711, Beckman).

Prostaglandin E_2 (Sigma Chemical Company, St. Louis) and terbutaline sulfate (Brethine, Taylor Pharmaceutical Co.) solutions were prepared from saline stock solutions on the day of each experiment. Prostaglandin E_2 was diluted with saline to a 12.5 μg/ml solution. Terbutaline was diluted with saline solution to yield two separate drug concentrations, 83.3 and 20 μg/ml.

Prostaglandin E_2 (22.4 ± 2.3 μg/min) was infused through the left ventricular catheter for 3 minutes. The dose was increased until a pressure response ≥5 mm Hg and uterine electric activity of at least 4 minutes' duration was elicited.[10]

When the appropriate prostaglandin E_2 dosage was determined, a second control challenge at the same dosage was repeated 30 minutes later. The responses to these two challenges were averaged to obtain a control value.

Seventeen minutes later, when the intrauterine pressure and uterine electric activity had returned to resting levels, terbutaline infusion was started. A loading dose was administered through the jugular vein at 8.5 μg/min for 3 minutes. A maintenance infusion was then given at 2 μg/min for the next 30 minutes. This dosage was selected from the low end of the dosage spectrum for clinical application.[5, 11]

Three more prostaglandin E_2 challenges were repeated in 30-minute intervals throughout the experiment. During terbutaline infusion, a challenge was given that began 20 minutes before the end of the infusion period and at 10 minutes and again at 40 minutes after the terbutaline infusion ended.

Data analysis. Data were read from the digital tape on a VAX VMS 8600 (Digital Equipment Corp., Maynard, Mass.) mainframe computer. The voltage readings were converted to digital pressure values from the electric signals recorded during the calibration procedure. Appropriate data segments were imported to an IBM XT (International Business Machines Corp., Boca Raton, Fla.) personal computer and stored on hard disks.

Polygraph records were examined to define the response period after each prostaglandin E_2 infusion. Five segments of data were extracted, including two control periods, terbutaline infusion, 10 minutes after the end of terbutaline treatment, and 40 minutes after the termination of infusion. Data segments were not of equal length. Segment length was determined by the duration of the prostaglandin E_2 response. If no response occurred, the average duration of the control responses was used to determine segment length. Uterine electromyogram and intrauterine pressure values were referred to the average values for the minute preceding each prostaglandin E_2 challenge.

Integrated responses were determined during each data segment, and each value was calculated as the sum of all activity that occurred in response to prostaglandin E_2 challenge minus the product of the sum of all activity that occurred in the 1-minute quiescent period before the response and the duration of the response to prostaglandin E_2. This calculation was performed to evaluate the combined effects of the magnitude and duration of each response and is expressed as the percent of the average control response. Fetal arterial pressure (referred to intrauterine pressure) and maternal arterial pressure were also determined during each response period. All data are expressed as mean ± SEM.

Statistical analysis was performed by two-way analysis of variance for repeated measures for the main effects of time versus measure (intrauterine pressure vs electromyogram response) on uterine activity. The Huynh-Feldt correction was applied throughout where appropriate.[12] Analysis of variance for repeated measures was used to examine the time-related effects of terbutaline infusion on maternal and fetal blood pressures. Differences between control and test values were tested by analysis of variance for contrast variables when the repeated-measures test was significant. Differences between intrauterine pressure and electromyogram responses were examined by the paired t test with the

Bonferroni correction for the critical value[18] when the interaction of time and measure in the two-way analysis of variance test was significant. For all tests, $p \leq 0.05$ was considered a significant reason to reject the null hypothesis.

Results

Studies were performed on five pregnant ewes at 130 ± 1 days' gestation. Maternal arterial pH (7.50 ± 0.01), P_{CO_2} (36.0 ± 1.7 mm Hg), P_{O_2} (110.0 ± 9.0 mm Hg), and hematocrit (29.5% ± 1.5%) were all within the normal range for this laboratory and indicate that the ewes were healthy and had recovered from surgery.

Maternal and fetal arterial pressure, intrauterine pressure, and uterine electromyogram were monitored continuously. Responses to prostaglandin E_2 challenge were quantitated before, during, and at 10 and 40 minutes after terbutaline infusion.

Neither maternal nor fetal arterial blood pressures demonstrated a significant change from control values at any time during the experiment. Fetal blood pressure (referenced to intrauterine pressure) averaged 50.3 ± 1.8 mm Hg during the control periods, and maternal blood pressure was 82 2 ± 3.9 mm Hg.

The contractile and electric responses to prostaglandin E_2 infusion were assessed by examination of the changes in intrauterine pressure and uterine electromyogram, respectively, during the control and terbutaline infusion periods and at 30-minute intervals after infusion. Two-way analysis of variance of the percent of control values for uterine electromyogram and intrauterine pressure responses to prostaglandin E_2 with time revealed a significant effect of time ($p \leq 0.0001$). This analysis also revealed a significant ($p \leq 0.05$) interaction between time and measurement (intrauterine pressure vs uterine electromyogram). These data are presented in Fig. 1 for both the intrauterine pressure and uterine electromyogram responses.

During the terbutaline infusion period the integrated intrauterine pressure response to prostaglandin E_2 infusion was 13.6% ± 5.5% of the average control value ($p \leq 0.0001$). The response remained significantly lower than that of control (47.2% ± 11.1%) during the prostaglandin E_2 challenge 10 minutes after the termination of the terbutaline infusion ($p \leq 0.01$). However, 40 minutes after the infusion period, the contractile response was not different from control (103.4% ± 16.9%).

During terbutaline infusion, electric activity, as measured by the uterine electromyogram response to prostaglandin E_2 challenge, fell to 7.8% ± 3.6% of the response elicited during the control period ($p \leq 0.0001$). During the 10- and 40-minute challenges, the integrated uterine electromyogram activity was not different from control (102.7% ± 17.9% and 108.9% ± 10.0%, respectively).

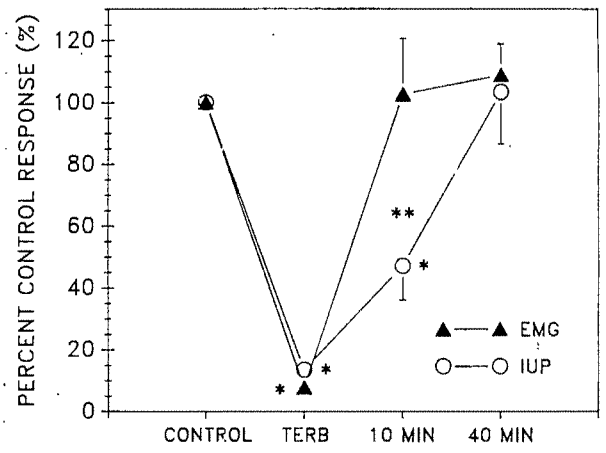


Fig. 1. Effect of terbutaline infusion ($n = 5$) on integrated uterine electromyogram *(EMG)* and intratuerine pressure *(IUP)* responses to prostaglandin E_2 challenge before *(control)*, during *(TERB)*, and at 10 minutes *(10 MIN)* and 40 minutes *(40 MIN)* after terbutaline infusion. *Asterisk*, Difference from control values ($p \leq 0.01$). *Two asterisks*, Differences between intrauterine pressure and electromyogram responses ($p \leq 0.01$) during single prostaglandin E_2 challenge. Data are shown as mean ± SEM.

When the paired t test was applied to each prostaglandin E_2 response, a significant difference was apparent between the uterine electromyogram and intrauterine pressure response to prostaglandin E_2 during the 10-minute challenge ($p \leq 0.01$). Although the integrated uterine electromyogram response was 102.7% ± 17.9% of control, the intrauterine pressure response was 47.2% ± 11.1% of control.

The responses of an individual animal throughout the experiment are shown in Fig. 2. The rise in intrauterine pressure and the increase in uterine electric activity in response to prostaglandin E_2 challenge during the control period are demonstrated in panel 1. Panel 2 shows the diminished electric and contractile activities during the terbutaline infusion period. In the third panel, which shows the response 10 minutes after infusion, the contractile response is still attenuated but the electric activity has already returned to control levels. Finally, panel 4 demonstrates that both variables returned to control values 40 minutes after infusion.

Comment

The effect of terbutaline sulfate infusion on the uterine response to prostaglandin E_2 stimulation was examined in five late-gestation ewes. The ewes were considered to be fully recovered from surgery as indicated by arterial blood gas tensions, pH, and feeding behavior. Prostaglandin E_2 was selected to stimulate uterine contractions because it can be given at discrete intervals

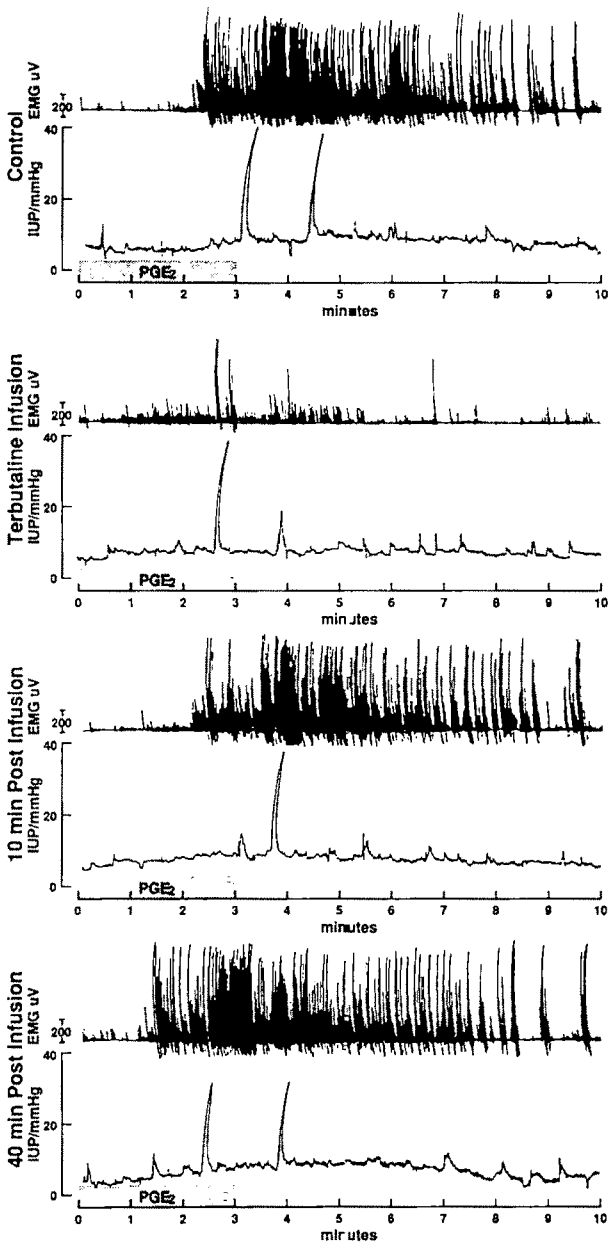


Fig. 2. Effect of terbutaline on electric and contractile responses to prostaglandin E_2 challenge in individual animal. Raw data are shown from control period, infusion period, and 10 and 40 minutes after terbutaline was infused. Uterine electromyogram is presented in *upper tracing*, and intrauterine pressure is shown in *lower tracing* in each panel.

whereas oxytocin challenge causes a protracted and unmanageable response.[8] Within 2 minutes of the beginning of a prostaglandin E_2 challenge, both the uterine electromyogram and intrauterine pressure responses are apparent; these responses are essentially simultaneous. These parameters usually return to baseline levels within 10 minutes of the termination of prostaglandin E_2 infusion. The prostaglandin E_2 stimulus can be repeated at regular intervals with no tachyphylaxis.[8]

This study reveals information that suggests a dual uterine response to the tocolytic properties of terbutaline. The early phase occurred during terbutaline infusion when both the contractile and electric responses to uterine stimulus were attenuated. The recovery phase appeared during the prostaglandin E_2 challenge administered 10 minutes after termination of the terbutaline infusion. The intrauterine pressure response to prostaglandin E_2 was approximately half the strength of the response before terbutaline treatment, and the magnitude of the uterine electromyogram signal was not different from the control values. These data indicate that the recovery time for contractility is slower than that for excitability when the intact ovine uterus is exposed to the tocolytic effects of terbutaline infusion.

In general, β-adrenergic receptors are thought to mediate smooth muscle relaxation by two pathways. First, the generation of spontaneous action potentials is suppressed by β-adrenergic receptor stimulation. For example, low concentrations of isoproterenol (4×10^{-7} mol/L) have been reported to cause cell membrane hyperpolarization that could be blocked by propranolol, as well as a fall in the membrane response to electric stimulus in isolated preparations of the pregnant rat myometrium.[14, 15] The intracellular mechanism for this action is not understood.[7] Second, the mechanical response to those action potentials generated is greatly reduced; that is, excitation-contraction coupling is weakened.[7] In vivo exposure of the myometrial cells of the gravid guinea pig to isoproterenol (1×10^{-5} mol/L) results in no effect on the membrane potential or membrane resistance in comparison with the evoked potentials before β-adrenergic receptor stimulation, although the contractile response was reduced to one half the intensity of the control responses.[16] This action may be mediated by cyclic adenosine monophosphate–stimulated increases in calcium sequestration, by increased calcium efflux, and/or by phosphorylation of the myosin light-chain kinase.[17] Thus, although the uncoupling between excitation and contraction in response to a β-adrenergic receptor agonist has not been previously reported in the intact pregnant uterus, this phenomenon has widespread support from the results of in vitro studies in the myometrium. We submit that attenuation of electric response and the uncoupling of excitation from contraction are separate effects of β-adrenergic receptor agonists that can exhibit different time courses.

We conclude that the uterine response to terbutaline tocolytic therapy in the gravid sheep consists of two phases. During infusion both electric and contractile

activity are diminished. In the recovery phase the electric response to prostaglandin E_2 is normal and contractile activity is depressed.

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Catecholamines modulate epidermal growth factor–induced prostaglandin E_2 production in amnion-like (WISH) cells by means of a cyclic adenosine monophosphate–dependent pathway

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OBJECTIVE: The purpose of this study was to test the hypothesis that catecholamines can modulate epidermal growth factor–induced prostaglandin E_2 production in amnion-derived cells via a cyclic adenosine monophosphate–dependent pathway.
STUDY DESIGN: Human amnion-derived WISH cells were used as the model system to study the regulation of prostaglandin E_2 production. The concentrations of prostaglandin E_2 and cyclic adenosine monophosphate were measured by radioimmunoassay. Statistical significance was determined with the Student *t* test.
RESULTS: Preexposure of WISH cells to either epinephrine, norepinephrine, or dopamine inhibited epidermal growth factor–induced prostaglandin E_2 production. In addition, propranolol blocked both the increase in cyclic adenosine monophosphate accumulation and the inhibition of prostaglandin E_2 production caused by epinephrine.
CONCLUSIONS: These results indicate that epidermal growth factor–induced prostaglandin E_2 production can be attenuated by preexposure of amnion cells to catecholamines and that the inhibitory effect of catecholamines on epidermal growth factor response may be mediated via a β-adrenergic receptor–coupled adenylate cyclase. (Am J Obstet Gynecol 1992;166:236-41.)

Key words: Amnion, catecholamines, epidermal growth factor (EGF), cyclic adenosine monophosphate (cAMP), prostaglandin E_2 (PGE_2), WISH cell

The mechanisms of human labor are not clearly understood, but prostaglandins (PGs) are thought to play a central role in the initiation and maintenance of cervical changes and myometrial contractions.[1-3] Several studies have shown that PGs (especially PGE_2 and $PGF_{2\alpha}$) found in the amniotic fluid increase with gestation and with the progression of labor.[4] Many investigators now believe that the amnion is a major source of amniotic fluid PGE_2.[1-3] The processes that regulate the production of PGs during labor are still undefined, but the signal for the initiation of labor is thought to emanate from the fetus.[5] One possible source of control is epidermal growth factor (EGF), which is a single-chain polypeptide of 6045 d that can stimulate the growth of a variety of tissues[6, 7] and may be secreted by the fetal kidney to provide a signal for initiating labor. Evidence that EGF may be important in human labor is based on the observation that the median concentration of EGF in amniotic fluid from women in labor is significantly higher than that in a control group of nonlaboring women.[8]

The importance of EGF in labor is further supported by the observation that EGF stimulates PGE_2 production by cultured amnion cells.[9] This effect of EGF does not preclude the possibility that other substances found in amniotic fluid may also influence PGE_2 biosynthesis in the amnion. Because the concentration of catecholamines increases in amniotic fluid during late pregnancy,[10, 11] these agents might also influence PGE_2 production. Our study was designed to test the hypothesis that catecholamines can modulate EGF-induced PGE_2 production by amnion cells by means of a β-adrenergic–coupled receptor, cyclic adenosine monophosphate (cAMP)–dependent pathway.

Material and methods

Cell cultures. WISH cells derived from amnion tissue were obtained from the American Type Culture Collection (ATCC CCL25). The cells were cultured in a mixture of Ham's F-12 and Dulbecco's modified Eagle medium (F12/DMEM) (1 : 1, vol/vol) with the addition

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6/1/32430

of 10% newborn calf serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, and 50 μg/ml gentamicin and were incubated at 37° C in 5% carbon dioxide and 95% air. The growth medium was changed every 2 days. When confluence was reached, the cell cultures were subcultured at lower density for use. Cells were plated in 24-well plates (2×10^5 cells per well) or 12-well plates (5×10^5 cells per well), and all experiments were conducted after 2-days growth, at which time the cells were 85% to 90% confluent.

Pretreatment studies. Cells growing in 24-well culture plates were rinsed once with 1 ml of serum-free F12/DMEM medium. The cells were then preincubated with 0.5 ml of growth medium in the presence or absence of test substances for 4 hours at 37° C. After the pretreatment phase, cells were treated with or without 10 ng/ml EGF for an additional 4 hours at 37° C. Thereafter the medium was collected and frozen at −70° C before PGE_2 determination.

PGE_2 determination. The PGE_2 concentration in the culture medium was determined by specific radioimmunoassay with antiserum prepared by inoculating domestic chickens with PGE_2–keyhole limpet hemocyanin conjugate. The details of the assay have been published elsewhere.[12]

cAMP accumulation. Cells growing in 12-well plates were rinsed once with 2 ml of F-10 medium (F12) with the addition of 15 mmol/L (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) and 1 mg/ml bovine serum albumin (F10-HEPES/BSA). The cells were then incubated with 1 ml of F10-HEPES/BSA medium in the presence or absence of test substances for the indicated time. The reaction was stopped by the addition of 2 ml 10% trichloroacetic acid into each well; the trichloroacetic acid solution was then collected and frozen at 70° C before cAMP determination.

cAMP determination. The trichloroacetic acid samples were extracted three times with a 1 : 3 (vol/vol) ratio of sample to water-saturated ethyl ether to remove trichloroacetic acid. Any remaining ether was removed by a 10-minute incubation in a 57° C water bath. cAMP concentration was then measured by a modification of the radioimmunoassay procedure of Steiner et al.[13]

Protein determination. Cell protein was measured by the method of Lowry et al.[14]

Statistical analysis. Statistical significance was determined with the unpaired, two-tailed Student *t* test. Data were presented as mean ± SEM of six replicates per condition.

Materials. All tissue culture reagents were from GIBCO (Grand Island, N.Y.). Human recombinant epidermal growth factor was from Upstate Biotechnology Institute (Lake Placid, N.Y.). [5,6,8,11,12,14,15-^{3}H(N)] PGE_2 was purchased from Amersham (Arlington Heights, Ill.). Iodine 125–cAMP was obtained from the laboratory of Dr. Sue O'Dorisio, Columbus Children's Hospital, Columbus, Ohio. cAMP antiserum was developed in our laboratory and is sensitive enough to measure 10 fmol cAMP. Epinephrine, (±)-propranolol, dibutyryl adenosine 3′:5′ cyclic monophosphate (dibutyryl cAMP), forskolin, and 3-isobutyl-1-methylxanthine (IBMX) were obtained from Sigma Chemical Company, St. Louis.

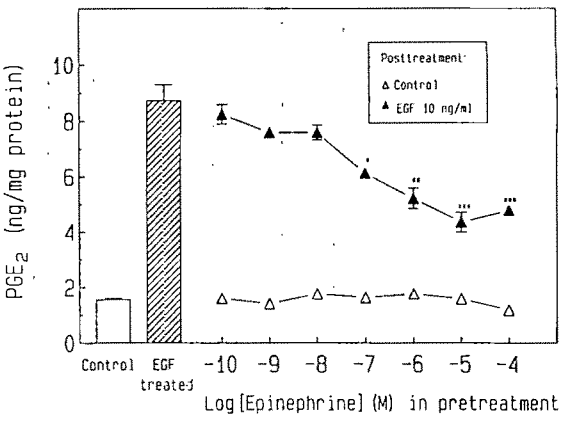


Fig. 1. Dose-dependent effect of epinephrine on EGF-induced PGE_2 production. Two days after plating, WISH cells were preincubated for 4 hours with indicated concentrations of epinephrine in F12/DMEM/10% newborn calf serum medium. After preincubation, cells were rinsed once with serum-free F12/DMEM medium and then exposed to F12/DMEM/10% newborn calf serum medium in presence or absence of 10 ng/ml EGF for an additional 4 hours. The concentration of PGE_2 in the medium was determined by radioimmunoassay. Data are presented as mean ± SEM of six replicates per condition from one of two separate experiments. (*Control,* Cells received medium only at both treatment phases; *EGF treated,* cells received medium at pretreatment phase and 10 ng/ml EGF at posttreatment phase. *One asterisk,* $p < 0.05$; *two asterisks,* $p < 0.001$; *three asterisks,* $p < 0.0001$; all compared with EGF treatment only.)

Results

Effect of catecholamines on EGF-induced PGE_2 production by amnion cells. Our initial results confirmed the earlier finding that EGF stimulates PGE_2 production by amnion cells in a time- and dose-dependent manner.[15, 16] In WISH cells the increase in PGE_2 production begins 1 hour after EGF treatment and reaches a maximum after a 4-hour exposure to EGF (data not shown). Under these conditions EGF at a concentration of approximately 10 ng/ml caused maximal stimulation approximately threefold to fourfold over control. On the basis of these results we conducted subsequent experiments with a concentration of 10 ng/ml EGF and a 4-hour incubation period. To test the hypothesis that catecholamines alter EGF-induced PGE_2 production in amnion cells, we incubated cells with various concentrations of catecholamines for 4 hours before an additional 4-hour exposure to EGF. Our results indicate that although exposure to concentrations of 10^{-10} to

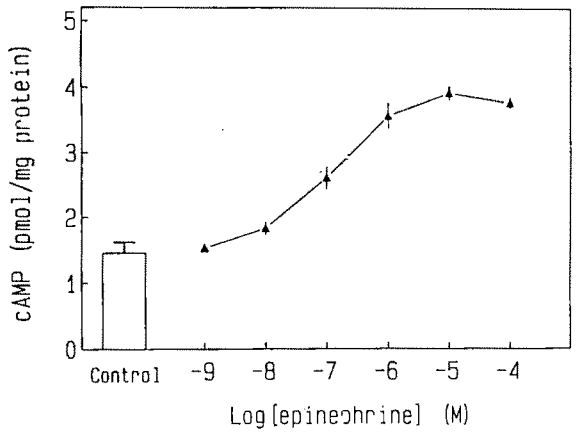


Fig. 2. Dose-dependent effect of epinephrine on cAMP accumulation in WISH cells. Two days after plating, WISH cells were incubated for 5 minutes with 5×10^{-5} mol/L IBMX and indicated concentrations of epinephrine in F10/HEPES/BSA medium. After 5-minute incubation, 10% trichloroacetic acid was added to plates to stop cAMP metabolism. cAMP concentration was determined by radioimmunoassay. Data represent mean ± SEM of six replicates per condition from one of two separate experiments. (*Control,* Cells received IBMX only.)

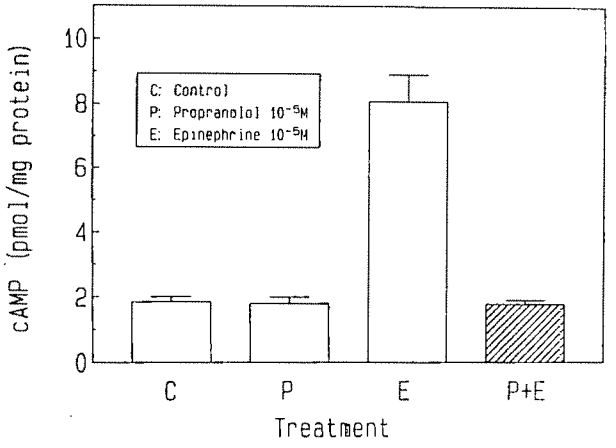


Fig. 3. Effect of propranolol on epinephrine-stimulated cAMP accumulation in WISH cells. Two days after plating, WISH cells were preincubated for 5 minutes with 10^{-4} mol/L IBMX in presence or absence of 10^{-5} mol/L propranolol in F10/HEPES/BSA medium. After preincubation phase, 10^{-5} mol/L epinephrine was added to some wells for an additional 5 minutes. After 5-minute incubation, 10% trichloroacetic acid was added to plates to stop cAMP metabolism. cAMP concentration was determined by radioimmunoassay. Data represent mean ± SEM of six replicates per condition. (*C,* Cells received IBMX only; *P,* cells received IBMX and propranolol; *E,* cells received IBMX and epinephrine; *P + E,* cells received IBMX, propranolol, and epinephrine.)

10^{-4} mol/L of epinephrine alone had little effect on PGE_2 production by WISH cells, EGF-induced PGE_2 production was inhibited by prior exposure of the cells to epinephrine (Fig. 1). Epinephrine at concentrations below 10^{-8} mol/L caused no significant effect on the EGF response. However, epinephrine at 10^{-7}, 10^{-6},

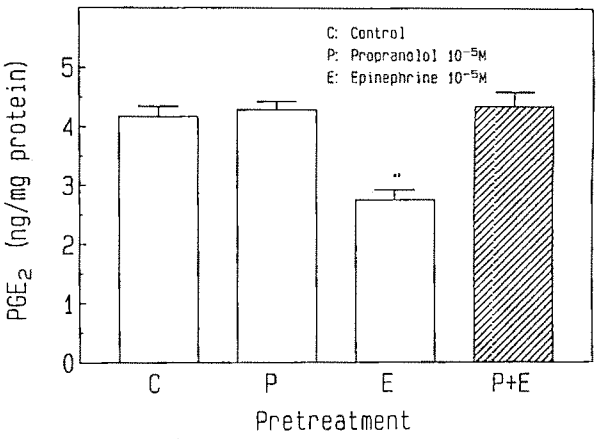


Fig. 4. Effect of propranolol on epinephrine-induced decrease in PGE_2 production in response to EGF. Two days after plating, WISH cells were preincubated for 30 minutes with or without 10^{-5} mol/L propranolol in F12/DMEM/10% newborn calf serum medium. After 30-minute incubation, 10^{-5} mol/L epinephrine was added to some wells for 3½ hours. After total 4-hour preincubation, cells were rinsed once with serum-free F12/DMEM medium and then exposed to 10 ng/ml EGF in F12/DMEM/10% newborn calf serum medium for an additional 4 hours. Concentration of PGE_2 in medium was determined by radioimmunoassay. Data represent mean ± SEM of six replicates per condition. (*C,* Control—cells received medium only in pretreatment period. All four groups received 10 ng/ml EGF in posttreatment period.)

10^{-5}, and 10^{-4} mol/L caused decreases of 30.1% ($p = 0.0012$), 40.6% ($p = 0.0003$), 50.4% ($p < 0.0001$), and 44.2% ($p < 0.0001$), respectively, in EGF-induced PGE_2 production. Similarly, norepinephrine and dopamine alone had little effect on PGE_2 production but inhibited EGF-induced PGE_2 production in a dose-dependent manner (data not shown). On the basis of these experiments we concluded that preexposure of amnion cells to catecholamines inhibits PGE_2 production in response to EGF.

Role of cAMP in inhibitory effect of catecholamines on EGF-induced PGE_2 production. Because cAMP has been shown to influence PG synthesis in a number of tissues,[17, 18] it is of interest to determine whether the inhibitory effect of catecholamines on EGF-induced PGE_2 production is dependent on cAMP accumulation. To test the hypothesis that the effect of catecholamines is the result of their ability to increase cAMP in WISH cells, WISH cells were exposed to epinephrine at concentrations ranging from 10^{-9} to 10^{-4} mol/L for 5 minutes. Our results indicate that epinephrine caused a dose-dependent increase in cAMP accumulation in WISH cells (Fig. 2). This increase was blocked by propranolol (Fig. 3). At a concentration of 10^{-5} mol/L, propranolol was able to completely eliminate the epinephrine-stimulated increase in cAMP accumulation.

From these experiments it is clear that the epinephrine-induced increase in WISH cell cAMP is by means

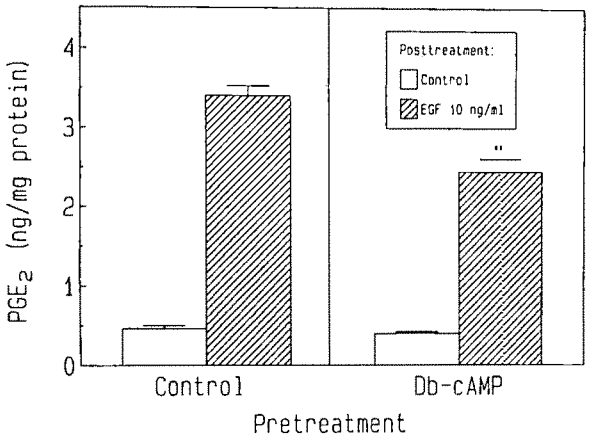


Fig. 5. Effect of cAMP analog on EGF-induced PGE_2 production. Two days after plating, WISH cells were preincubated for 4 hours with or without 5×10^{-5} mol/L dibutyryl cAMP in F12/DMEM/10% newborn calf serum medium. After preincubation phase, cells were rinsed once with serum-free F12/DMEM medium and then exposed to F12/DMEM/10% newborn calf serum medium in presence or absence of 10 ng/ml EGF for additional 4 hours. Concentration of PGE_2 in medium was determined by radioimmunoassay. Data represent mean ± SEM of six replicates per condition from one of two separate experiments. (*Control*, Cells received vehicle only.)

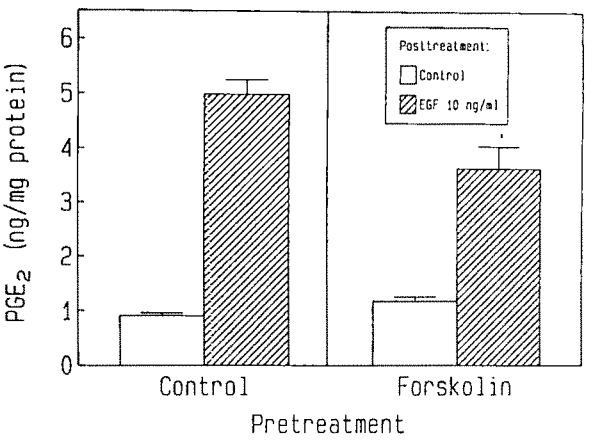


Fig. 6. Effect of adenylate cyclase activator on EGF-induced PGE_2 production. Two days after plating, WISH cells were preincubated for 4 hours with or without 5×10^{-5} mol/L forskolin in F12/DMEM/10% newborn calf serum medium. After preincubation phase, cells were rinsed once with serum-free F12/DMEM medium and then exposed to F12/DMEM/10% newborn calf serum medium in presence or absence of 10 ng/ml EGF for additional 4 hours. Concentration of PGE_2 in medium was determined by radioimmunoassay. Data represent mean ± SEM of six replicates per condition from one of two separate experiments. (*Control*, Cells received vehicle only.)

of the β-adrenergic receptor. We next determined the effect of propranolol on the epinephrine-induced decrease in PGE_2 production. WISH cells were exposed to propranolol 30 minutes before epinephrine pretreatment. Our results indicate that 10^{-5} mol/L propranolol was able to block the inhibitory effect of epinephrine on EGF-induced PGE_2 production (Fig. 4). This is in accord with the previous result that propranolol at the same concentration eliminated the stimulatory effect of epinephrine on cAMP accumulation. Because propranolol blocked both the increase in cAMP accumulation and the inhibition of PGE_2 production that resulted from epinephrine, we conclude that the inhibitory effect of epinephrine on EGF-induced PGE_2 production may be mediated by means of an adenylate cyclase–coupled β-adrenergic receptor.

The effect of cAMP-stimulating agents on EGF-induced PGE_2 production. To confirm that the inhibitory effect of catecholamines on PGE_2 production in response to EGF was by means of a cAMP-dependent pathway, WISH cells were pretreated with a cAMP analog (5×10^{-5} mol/L dibutyryl cAMP), an adenylate cyclase activator (5×10^{-6} mol/L forskolin), or a phosphodiesterase inhibitor (5×10^{-5} mol/L IBMX) for 4 hours before EGF exposure. Our results indicate that all three agents reduced PGE_2 production in response to EGF (Figs. 5 to 7). As with epinephrine, none of these three cAMP-stimulating agents alone had a significant effect on PGE_2 production. On the basis of these experiments, we conclude that cAMP caused a

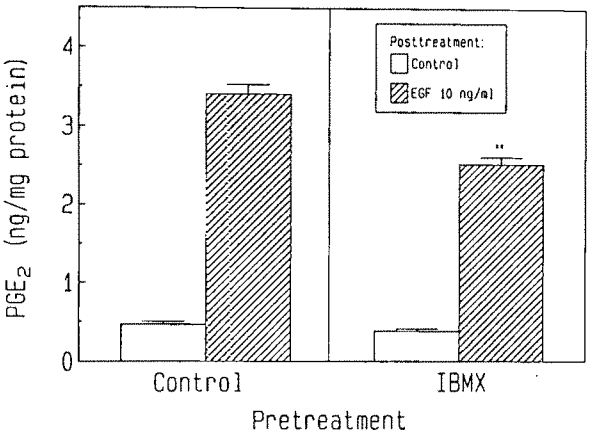


Fig. 7. Effect of phosphodiesterase inhibitor on EGF-induced PGE_2 production. Two days after plating, WISH cells were preincubated for 4 hours with or without 5×10^{-5} mol/L IBMX in F12/DMEM/10% newborn calf serum medium. After preincubation phase, cells were rinsed once with serum-free F12/DMEM medium and then exposed to F12/DMEM/10% newborn calf serum medium in presence or absence of 10 ng/ml EGF for additional 4 hours. Concentration of PGE_2 in medium was determined by radioimmunoassay. Data represent mean ± SEM of six replicates per condition from one of two separate experiments. (*Control*, Cells received vehicle only.)

decrease in EGF-induced PGE_2 production. Our results suggest that preexposure of amnion cells to catecholamines inhibits EGF-induced PGE_2 production and that this inhibitory effect may be by means of β-adrenergic receptor–coupled adenylate cyclase.

Comment

Human amnion has been proposed to be a target tissue for labor-initiating signals emanating from the fetus.[3, 19] It is also considered to be a major source of PGs (primarily PGE_2) produced by intrauterine tissue at term.[20, 21] Because the synthesis and metabolism of PGs in human amnion cells grown in monolayer cultures are similar to those in amnion tissue,[22, 23] these cells are useful as a model for the study of the regulation of PG synthesis. Although primary amnion cell cultures are very useful and have been used by several laboratories, different culture preparations may vary considerably. In this study we used a human amnion cell line (WISH) as the model system with which to investigate the regulation of PGE_2 production. Recent studies from our laboratory[24] suggest that WISH cells are an effective model for the study of EGF-induced PGE_2 production. In addition, a study by Harris et al.[16] also used WISH cells to investigate amniotic PGE_2 production.

Previous studies have identified EGF receptors in many tissues, including fetal membranes[25] and human amnion (WISH) cells.[26] In addition, EGF alters amnion function by increasing PGE_2 production in a time- and dose-dependent manner.[15, 16] Because concentrations of catecholamines increase in amniotic fluid during late pregnancy[10, 11] and because there are functional β-adrenergic receptors in human amnion tissue,[27] it is reasonable to hypothesize that EGF plays a critical role in the initiation of human labor by stimulating PGE_2 production in amnion cells and that this effect can be modulated by catecholamines. In fact, our results indicate that, although preexposure of amnion cells to catecholamines alone does not alter PGE_2 production, these agents do inhibit EGF-induced PGE_2 production. Because propranolol attenuates this inhibitory response, the effect is probably mediated via a β-adrenergic receptor. However, a previous study by Warrick et al.[17] reported that β-adrenergic receptor agents and cAMP-stimulating agents stimulate PGE_2 output from dispersed cells of amnion tissues. Their observation differs both from our finding and from the study by Casey et al.,[15] which showed that dibutyryl cAMP and isoproterenol fail to trigger PGE_2 production in primary human amnion cell culture. This suggests that the observed difference between our finding and that of Warrick et al. is not solely due to our use of the WISH cell line. Our observation that β-adrenergic receptor agents can decrease the production of the labor-inducing factor (PGE_2) is in accord with previous observations that β-adrenergic receptor agonists can inhibit uterine activity in preterm labor.[28]

In this study we have provided evidence to support a central role for EGF and a modulating role for catecholamines in the control of PGE_2 production by amnion-derived cells in vitro. Our results suggest that in the human amnion cell line (WISH) catecholamines stimulate the accumulation of cAMP, which, in turn inhibits PGE_2 production in response to EGF. This indicates that the EGF-induced PGE_2 biosynthesis pathway is negatively modulated by adenylate cyclase. Because catecholamines only partially inhibit the EGF effect, it is likely that PGE_2 production is influenced by other factors as well. For example, our previous studies[24] have shown that activators of protein kinase C augment EGF-induced PGE_2 production in amnion cells. This suggests the possibility of a complex link between adenylate cyclase and protein kinase C in amnion cells.

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Circulating hyaluronic acid in nonpregnant, pregnant, and postpartum guinea pigs: Elevated levels observed at parturition

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OBJECTIVE: Dilatation of the uterine cervix at parturition is associated with an increase in cervical hyaluronic acid content. The objective is to test the hypothesis that circulating hyaluronic acid is increased at parturition.
STUDY DESIGN: Serum hyaluronic acid levels from nonpregnant (n = 5), pregnant (n = 13), and postpartum (n = 4) adult Hartley guinea pigs were determined with a radiometric assay that utilizes iodine 125–labeled hyaluronic acid–binding protein. Results were analyzed for statistical significance with Student's paired t test and regression analysis.
RESULTS: The serum hyaluronic acid level in nonpregnant animals was 238 ± 88 ng/ml (mean ± SEM). During pregnancy, serum hyaluronic acid levels were 127 ± 12 and 126 ± 34 ng/ml at 25 and 50 to 63 days' gestation, respectively. At parturition, hyaluronic acid levels increased fivefold to 765 ± 111 ng/ml ($p < 0.001$). Hyaluronic acid levels returned to antepartum values 2 days post partum (153 ± 27 ng/ml). There was no significant difference between arterial and venous levels.
CONCLUSION: Circulating hyaluronic acid levels increase significantly at parturition in the guinea pig.
(AM J OBSTET GYNECOL 1992;166:242-6.)

Key words: Hyaluronic acid, parturition, guinea pig

Hyaluronic acid (hyaluronan) is a linear polysaccharide of high molecular weight (1 to 4 × 10^6 d), which is widely distributed throughout the body. Its voluminous structure and its inherent stiffness resulting from its molecular size and hydration result in a profound viscoelasticity that may be important in structural integrity and function of connective tissues.[1] It is synthesized at the plasma membrane by a membrane-bound hyaluronic acid synthetase.[2] It is present in the tissue as a coat attached to cell surface, as a scaphoid for aggregation of proteoglycans, or as a free polymer.[3] It enters the circulation by lymphatic drainage and is endocytosed by endothelial cells of liver, where it is digested.[4] Elevated circulating levels of hyaluronic acid are present in liver cirrhosis (decreased removal), rheumatoid arthritis, scleroderma, and cancer (increased production).[5-8]

The biologic function of hyaluronic acid in the body is poorly understood. It modulates many cellular functions such as cell migration,[9] invasion,[10, 11] proliferation,[12, 13] transformation,[14] and mitosis.[15] A wide variety of agents have been shown to increase hyaluronic acid synthesis in culture, including insulin, testosterone, prostaglandins, interleukin-1, cyclic adenosine monophosphate, lipopolysaccharides, platelet-derived growth factor, and epidermal growth factor. Hyaluronic acid synthesis is inhibited by glucocorticoids, estrogen, and adenosine.[3]

Pregnancy is a state of active cellular growth and differentiation in the fetus, accompanied by marked remodeling of maternal reproductive organs. Perhaps the most dramatic change in tissue structure in the mother is the rapid dilatation of the uterine cervix at parturition. The cervix is regarded as a fiber-reinforced composite viscoelastic material, because it is made of elastic solid (collagen) and viscous macromolecules (proteoglycans).[16, 17] Stress-strain studies have shown that in the nonpregnant rat the maximum possible dilatation of the cervix is a 50% increase in internal diameter without rupture,[18] accompanied by an overall reduction in strength and stiffness at parturition (in the rat) of the order of 20-fold to 40-fold.[17] However, the cervix in all mammals undergoes a severalfold increase in diameter at parturition without rupture. This remarkable alteration in the biomechanical properties of the cervix can only be explained by a major change in its biochemical composition.

Biochemical changes in the cervix at parturition include a 50% reduction in collagen content, a twofold increase in hyaluronic acid, and a sixfold to 14-fold increase in collagenase, accompanied by active degra-

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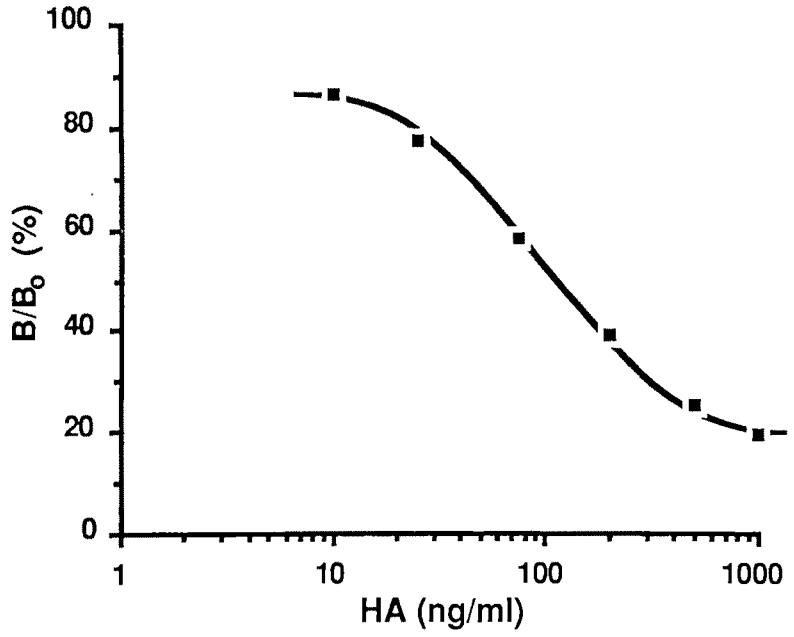


Fig. 1. Hyaluronic acid *(HA)* standard curve. *B/Bo (%)* represents percent of activity bound that is obtained by dividing counts *(B)* for standards as percentage of mean counts in absence of hyaluronic acid *(Bo)*. Hyaluronic acid concentrations are plotted on semilog scale.

dation of type I collagen (the predominant collagen in the cervix) in the extracellular matrix in women and in the guinea pig.[19-22] The marked increase in cervical collagenase at delivery is associated with a significant increase in circulating collagenase at term and in premature deliveries in women.[23] We hypothesized that parturition may be accompanied by a similar increase in circulating hyaluronic acid in the guinea pig. This study confirms this hypothesis.

Material and methods

Serum. Blood from anesthetized animals (see below) was collected by intracardiac puncture. Serial arterial and venous blood samples were simultaneously collected by withdrawing 1 ml aliquots from jugular and carotid catheters. The blood was allowed to clot for 30 minutes at room temperature. Serum was separated by centrifugation 365*g* for 10 minutes at 4° C and frozen at −20° C until analyzed for hyaluronic acid.

Animals. Adult Hartley guinea pigs (Charles River Breeding Laboratories, Montreal) were housed in guinea pig cages (three to five animals per cage) and fed guinea pig chow and water as described. All pregnant animals were of known gestational ages. Timed pregnancies were induced by mating animals during the fertile period immediately post partum.

Surgical procedure. Animals were equipped with indwelling vascular catheters as follows. Guinea pigs were catheterized while they were under general anesthesia with ketamine (40 mg/kg) and xylazine (1 mg/kg) intramuscularly. The shoulder and ventral neck regions were clipped, and the jugular vein and carotid artery were exposed aseptically. Vascular catheters were constructed of 0.02 × 0.06 Tygon microbore tubing (Norton Plastics, Akron, Ohio) with a tension-fit intravascular tip (1 cm) of 0.58 × 0.96 mm vinyl tubing (Dural Plastics, Dural, New South Wales, Australia). Catheters were funneled subcutaneously to the shoulder where approximately 5 cm of tubing protruded from the skin. The neck incision was closed, and patency of the catheters was maintained by daily flushing with saline solution. The dead space of the catheters was filled with heparin (1000 U/ml of saline solution) at the end of each flushing, and the catheters were sealed with a stainless steel wire plug.

Hyaluronic acid assay. A radiometric assay for hyaluronic acid determination was used (Pharmacia HA test 50) as described by the manufacturer. Hyaluronic acid standards (10 to 1000 ng/ml) or serum samples (25 to 100 μl) were incubated in duplicate with iodine 125–hyaluronic acid binding protein for 60 minutes at room temperature. The unbound ^{125}I-hyaluronic acid binding protein was removed by incubation with hyaluronic acid–Sepharose for 45 minutes. After addition of 2 ml of the washing solution, the mixture was centrifuged at 2000*g* for 10 minutes. This washing step was repeated and the supernatant was discarded. The radioactivity of the precipitate was measured in a γ-counter.

Calculation of results. The counts (B) for standards and unknown were expressed as a percentage of the mean counts of the hyaluronic acid–free standard (Bo).

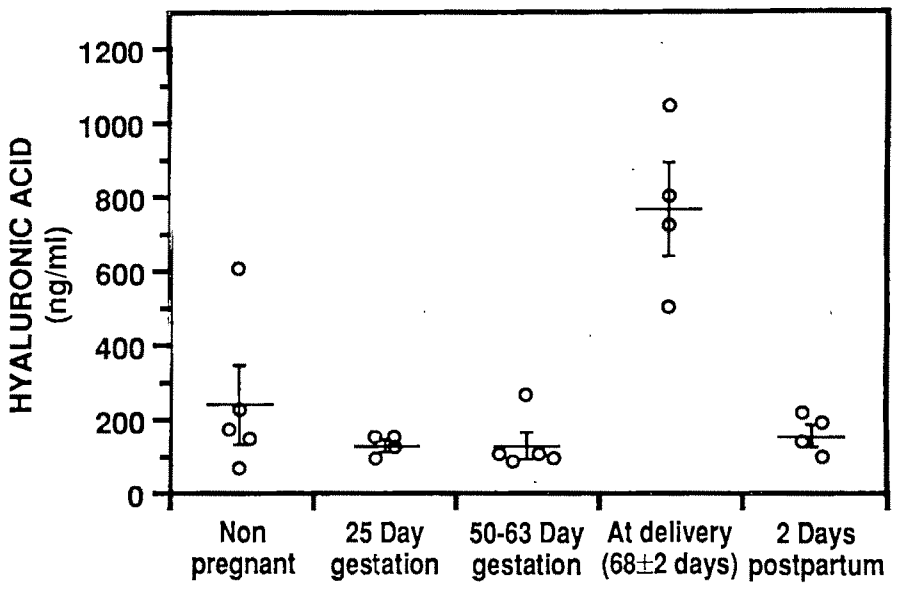


Fig. 2. Hyaluronic acid *(HA)* levels in sera from cardiac puncture were assayed as described in Material and Methods. *Horizontal lines,* Means; *vertical lines,* standard error of mean. $p < 0.001$ at delivery versus 25 to 63 days' gestation.

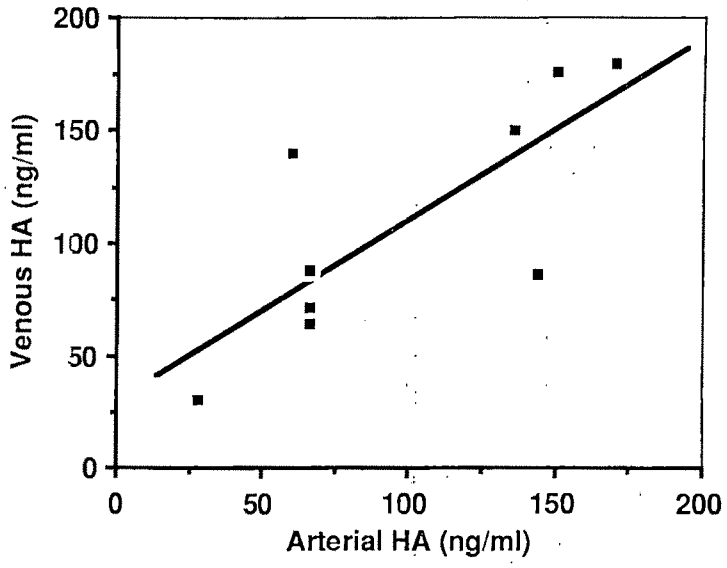


Fig. 3. Distribution of hyaluronic acid *(HA)* in circulation of guinea pigs that were 50 days pregnant. Venous hyaluronic acid = 121% ± 5.7% compared with arterial hyaluronic acid (mean ± SEM, $n = 9$, not statistically significant). Correlation coefficient between two vascular compartments is 0.77.

Percent activity bound = (B × 100)/Bo. The percentage binding for the standards was plotted against the hyaluronic acid concentration, and a standard curve was constructed (Fig. 1). The concentration of the unknown samples was read from the standard curve. The lower limit of detection for guinea pig serum of the assay is 10 ng/ml of serum. Coefficient of variation was 11% between assays and 9% within assays.

Statistical analysis. The significance of differences between mean values were determined by the Student paired t test. A value of $p \leq 0.05$ was considered significant. Regression analysis was used to examine the correlation between venous and arterial blood samples.

Results

Circulating hyaluronic acid. Concentrations of hyaluronic acid are shown in Fig. 2. The level in nonpregnant animals decreased during pregnancy. Low

levels continued to be present until day 63 of gestation. At parturition, there was a significant fivefold increase in hyaluronic acid levels ($p < 0.001$). Levels 2 days post partum returned to antepartum levels.

Distribution of hyaluronic acid in the circulation. The correlation between levels in arterial and venous samples is presented in Fig. 3 ($r = 0.77$). Venous samples contained more hyaluronic acid than did arterial samples (mean ± SEM 121% ± 5.7%, $n = 9$). This difference, however, was not statistically significant.

Comment

In this article we report on the changes in the level of circulating hyaluronic acid in the guinea pig pregnancy and at parturition. Nonpregnant animals have much higher levels than previously reported for nonpregnant healthy women.[6] This may be due to species differences. Levels during pregnancy did not change significantly from day 25 to day 63 of gestation. Hyaluronic acid during pregnancy may play a role in the growth and development of the placenta.[24] Of significance is the fivefold increase at delivery. Circulating hyaluronic acid has a half-life of 2.5 to 5 minutes because of its rapid uptake and metabolism in the liver.[3] The marked increase in steady-state levels at birth represents either a decrease in removal or an increase in production or mobilization from the extracellular matrix. There is no evidence that liver function is impaired at parturition. Thus the increase in hyaluronic acid probably results from an increase in production or mobilization from the extracellular matrix into the circulation by lymphatic drainage. Although the origin of hyaluronic acid in the circulation is not known, the observed increase at parturition may result in part from the increased content or mobilization of hyaluronic acid from the extracellular matrix of the cervix and the lower uterine segment at parturition.

In ovariectomized pregnant rats (day 18 to 22 of gestation) treated with progesterone and 17β-estradiol, the administration of highly purified porcine relaxin resulted in a significant increase in cervical hyaluronic acid content.[25] The surge of hyaluronic acid at parturition may be caused by an increase of relaxin at that time. Prostaglandins have been reported to increase hyaluronic acid biosynthesis; therefore it is possible that the observed increase at birth may be mediated by an increase in prostaglandins.[8] An accumulation of hyaluronic acid in the extracellular matrix may result in the observed softening and swelling of the organ because of its unique viscoelastic properties and its high avidity for water. An increased content in the cervix accompanied by active collagen degradation by collagenase[21-23] is associated with a marked alteration of the structural rigidity and tensile strength of the cervix leading to rapid cervical dilatation. The source of circulating hyaluronic acid and its role in the process of cervical dilatation, however, remain to be determined.

We thank Jane Wishart and Mark Lepick for processing the figures.

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Change in electrocardiogram T-wave amplitude during umbilical cord compression is predictive of fetal condition in sheep

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OBJECTIVE: The purpose of this study is to assess the usefulness of the dynamic change in T/QRS ratio in fetal electrocardiograms in predicting the fetal condition when repetitive variable decelerations are seen in intrapartum cardiotocograms.
STUDY DESIGN: We investigated the relationship, using linear regression and Wilcoxon's test, between T/QRS and blood gas values, catecholamine concentrations, and blood pressure during repetitive cord compression in five chronically instrumented lamb fetuses.
RESULTS: T/QRS during cord compression correlated significantly ($p < 0.01$) with fetal arterial pH ($r = -0.7711$), norepinephrine concentration ($r = 0.7551$), and duration of elevated blood pressure during compression ($r = -0.8619$). Fetal arterial pH and base excess were lower, the duration of elevated blood pressure during compression was shorter, and carbon dioxide partial pressure and catecholamine concentrations were higher in the stage with higher (>0.50) T/QRS during compression ($p < 0.005$).
CONCLUSION: We can estimate the severity of fetal distress by measuring T/QRS near the bottom of the decelerations. (Am J Obstet Gynecol 1992;166:246-55.)

Key words: Fetal electrocardiogram, variable deceleration, umbilical cord compression, fetal acidemia, catecholamines

Today fetal heart rate (FHR) monitoring is widely used for intrapartum surveillance. Among the various patterns seen during FHR monitoring, variable decelerations are frequently observed during labor[1] and are considered to be caused by umbilical cord compression.[2,3] In the clinical setting, fetal distress is diagnosed on the basis of the depth and duration of the variable decelerations.[2,4] However, we sometimes encounter the case in which fetal compromise is not predictable by either the depth or the duration of the variable decelerations alone.[5-7] Therefore additional or alternative methods are necessary to evaluate fetal acidemia when repetitive variable decelerations are seen repeatedly in intrapartum FHR monitoring.

It is reported that fetal hypoxemia induced by allow-

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ing the ewe to breathe a low-oxygen mixture[8-10] or by reducing the aortic blood flow of the ewe[11, 12] results in a progressive increase in T-wave amplitude in the fetal electrocardiogram (ECG). However, change in T-wave amplitude during umbilical cord compression and its alteration as fetal acidemia develops has not been reported.

In this study we attempted to induce decelerations in the FHR recording by repeated umbilical cord compression using chronically instrumented lamb fetuses. We then observed the change in T-wave amplitude, as the T/QRS ratio, in the fetal ECG during cord compression and the ways in which it altered as fetal acidemia developed. The relationship between T/QRS and fetal arterial gas values, catecholamine concentrations, and fetal blood pressure during compression were also investigated during repeated cord compression.

The aim of the current study was to assess the usefulness of the dynamic change in T/QRS in fetal ECG in predicting fetal arterial gas values, blood pressure, and catecholamine concentrations when repetitive variable decelerations are seen in FHR monitoring.

Material and methods

The experiments were performed on five chronically instrumented lamb fetuses of known gestational age, ranging between 125 and 134 days (mean 130.0 days), carried by ewes of mixed breed. After the animals fasted for at least 24 hours, a midline abdominal incision was made with the ewes under general anesthesia (nitrous oxide and halothane) with tracheal intubation. After exteriorization of the fetus, polyvinyl catheters (outer diameter 2.0 mm, inner diameter 1.2 mm) were inserted aseptically into the fetal carotid artery and jugular vein with the animals under local anesthesia. Tripolar cardioelectrodes were stitched subcutaneously to the bilateral axilla and the right hind leg of the fetus. A vascular occluder with an inflatable balloon (OC-16, In Vivo Metric Inc., Healdsburg, Calif.) was placed around the umbilical cord near the fetal abdomen. After surgical procedures on the fetus had been completed, the fetus was returned to the uterus, and a polyvinyl catheter was inserted into the amniotic cavity before closure of the uterus and abdominal wall of the ewe. The catheters and cables were led directly out from the incision wound. The ewe's activity, food and water intake, and urine and excrement production were assessed daily. The arterial catheter was infused continuously with heparin solution (0.1 IU/ml) to maintain patency of the catheter. Antibiotics (mainly ABPC) were administered into the maternal vein, amniotic cavity, and fetal vein daily during the postoperative period.

The experiments were performed after the fourth postoperative day. We designed the repetitive cord compression, as shown in Fig. 1, to induce gradually developing fetal acidemia. The cord was compressed by inserting sterile saline solution into the balloon of the occluder for 40 seconds, followed by a period of 80 seconds' release. We performed this set of procedures 15 times and followed it with 60 seconds of compression and 60 seconds of release for zero (two experiments), five (two experiments), and 10 (one experiment) repetitions. The number of compressions in each experiment ranged from 15 to 25 (mean 20), and the total duration of each experiment was 30 to 50 minutes. Umbilical arterial blood flow completely ceased within 3 to 4 seconds after the beginning of saline solution insertion, as confirmed by measuring umbilical arterial blood flow with an ultrasonographic transit-time flowmeter (T101 Transonic Systems Inc., Ithaca, N.Y.).[13] Samples of 0.6 to 0.8 ml of blood were taken before the experiments and immediately after the release of every fifth cord compression. Blood gases were analyzed with an AVL 940 system (AVL AG, Schaffhausen, Switzerland) with temperature correction to 39° C. Base excess was calculated with this system. After blood gas analyses, blood plasma was separated by centrifugation and stored frozen at −80° C until assayed.

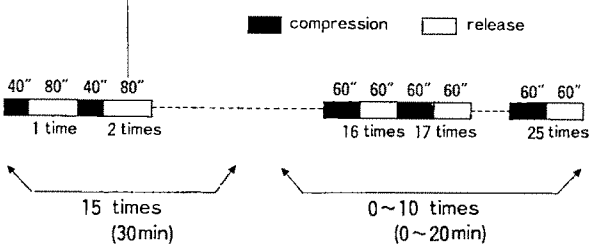


Fig. 1. Experimental procedure for repeated umbilical cord compression.

Plasma norepinephrine and epinephrine levels were measured in 50 μl plasma samples by means of the single-isotope radioenzymatic assay, as modified by Peuler and Johnson.[14] The sensitivity was 40 pg/ml for norepinephrine and 28 pg/ml for epinephrine. The assay was linear for values up to 20,000 pg/ml, with a slight decrease in linearity from 20,000 to 60,000 pg/ml. The within-run coefficient of variation did not exceed 14%. Reproducibility was monitored by inclusion of a plasma pool (norepinephrine 357 ± 27 pg/ml, epinephrine 28 ± 7 pg/ml; mean ± sd).

FHR was monitored with a cardiotachometer triggered by the cardioelectric signal. Fetal arterial pressure was measured with pressure transducers (model P23ID, Gould, Inc., Oxnard, Calif.; model 4-327-I, Bell & Howell, Inc., Pasadena, Calif.) and was corrected for intrauterine pressure. Fetal ECG (obtained by unipolar limb lead, similar to the aVF lead in humans), heart

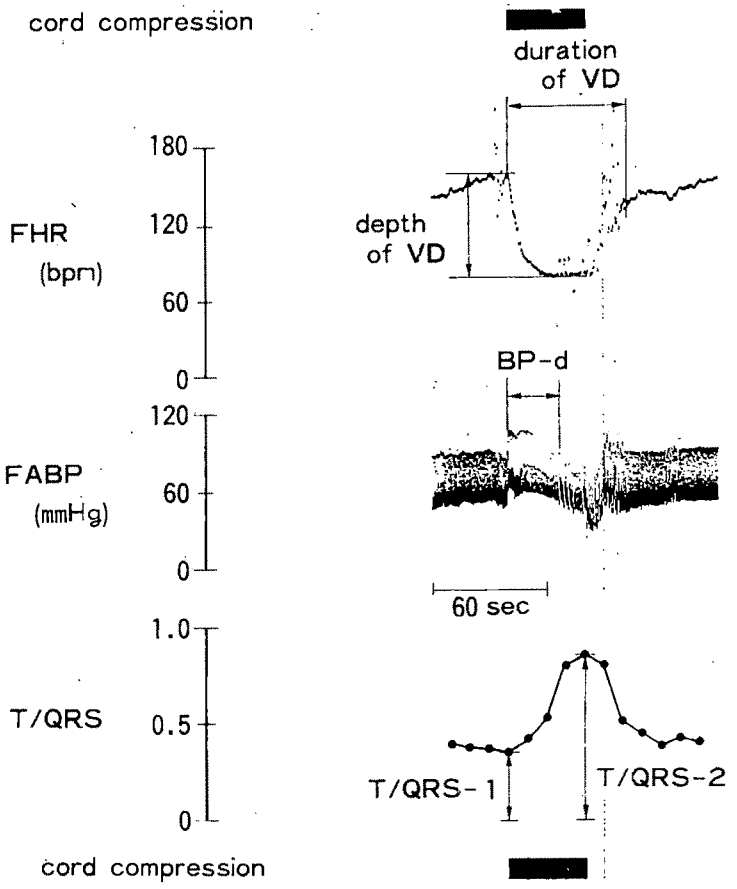


Fig. 2. Measurements of duration of elevated fetal blood pressure *(BP-d)* and T/QRS ratio from fetal ECG. *T/QRS-1,* T/QRS just before compression; *T/QRS-2,* T/QRS during (40 seconds after start of) compression.

rate, and fetal arterial pressure were recorded continuously on a pen recorder (Recti-Horiz 8K NEC, Sanei Instruments Co., Ltd., Tokyo) and data recorder (A49 Sony Magnescale Inc., Tokyo).

Changes in the T wave of the fetal ECG were expressed as the ratio of the amplitude of the T wave to that of the QRS complex (T/QRS). T/QRS was measured just before (T/QRS-1) and at the point 40 seconds after the start (T/QRS-2) of every fifth compression (Fig. 2). Duration of elevated blood pressure during cord compression was measured from the onset of the rise in systolic blood pressure immediately after the start of cord compression until the systolic pressure decreased to the precompression value. Correlations of T/QRS (both T/QRS-1 and T/QRS-2) with fetal arterial gas values, catecholamine concentrations, and duration of elevated blood pressure during compression were investigated.

In analyzing the data, the mean and standard deviation were calculated. Wilcoxon's rank sum test was used to calculate statistical significance. Correlations of T/QRS with other variables (gas values, catecholamine concentrations, and duration of elevated blood pressure during cord compression) were derived by linear regression.

Results

Five experiments were performed on five fetuses. Mean fetal arterial pH before the experiment was 7.303 (range 7.262 to 7.342). Mean plasma norepinephrine and epinephrine concentrations at the start of experiments were 1142 (range 690 to 1906) and 121 (range 17 to 296) pg/ml, respectively.

Fig. 3 shows the change in fetal arterial gas values during the experiments. Fetal arterial pH and base excess decreased gradually during the experiment. P_{O_2} dropped first and changed little after that. P_{CO_2} increased gradually in most cases.

Although the plasma norepinephrine and epinephrine levels continued to rise up to the fifteenth or twentieth compression, thereafter they reached a plateau at a pH of around 7.15 (Fig. 4).

Fig. 5 shows how FHR pattern, fetal arterial pressure, and T/QRS in fetal ECG altered as fetal acidemia de-

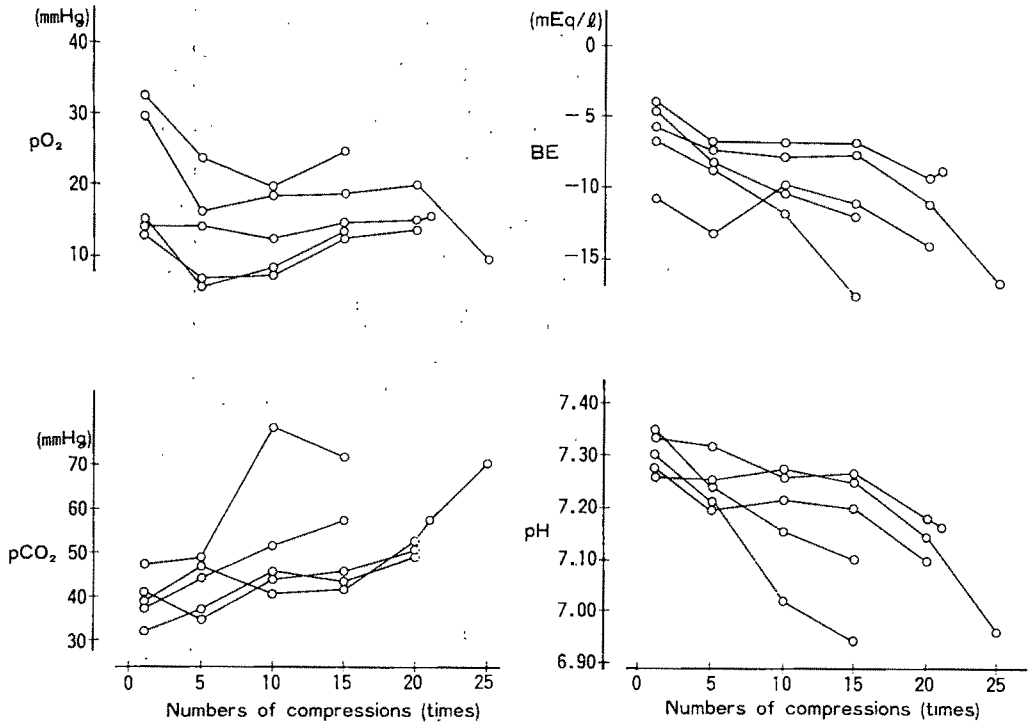


Fig. 3. Change in fetal arterial P_{O_2}, P_{CO_2}, base excess *(BE)*, and pH during experiment.

veloped. By compressing the umbilical cord repeatedly, repetitive deceleration could be observed on FHR monitoring. Each deceleration started a few seconds after the start of cord compression and recovered gradually after release of the compression. The depth and duration of decelerations remained almost unchanged as the fetus became acidotic. At the beginning of the experiment, fetal arterial blood pressure increased suddenly, immediately after the start of cord compression, and persisted during the compression period. However, as the fetus became acidotic, fetal arterial blood pressure gradually dropped during the compression period. T/QRS was measured every 10 seconds and plotted on the chart. T/QRS did not change much during cord compression at the beginning of the experiment. However, as fetal acidemia developed, T/QRS increased gradually after the start of cord compression and returned to the precompression value with the release of compression, just the mirror image of FHR deceleration. Further, this change in T/QRS during cord compression became more prominent as fetal acidemia progressed further.

We investigated the changes in T/QRS at two points, one just before the compression (Fig. 2, T/QRS-1) and another 40 seconds after the start of compression (T/QRS-2), as mentioned previously. In FHR recording, these points are just before the onset of deceleration (T/QRS-1) and during deceleration (T/QRS-2). Both T/QRS-1 and T/QRS-2 increased gradually during the experiment (Fig. 5).

Fig. 6 shows the relationship between T/QRS-2 and fetal arterial pH. There was a negative linear significant correlation between them ($r = -0.7711$, $p < 0.01$). The relationships between T/QRS-2 and P_{CO_2} and duration of elevated blood pressure during cord compression are shown in Fig. 7 ($r = 0.7383$, $p < 0.01$) and in Fig. 8 ($r = -0.8619$, $p < 0.01$), respectively. In Fig. 9 the relationship between duration of elevated blood pressure during cord compression and pH is presented. There were strong correlations between these variables ($r = -0.9024$, $p < 0.01$). Other calculated correlation coefficients for T/QRS versus fetal variables (arterial gas values, plasma catecholamine concentrations, and duration of elevated blood pressure during cord compression) are listed in Table I. T/QRS correlated well with pH, P_{CO_2}, base excess, norepinephrine concentrations, and duration of elevated blood pressure during cord compression. No significant correlation was found between T/QRS and P_{O_2}. Duration of elevated blood pressure during cord compression correlated strongly with pH, as mentioned above.

Sensitivities, specificities, and predictive values in prediction of fetal acidemia by T/QRS are calculated and listed in Table II. Table III shows the difference in fetal arterial gas values, catecholamine concentra-

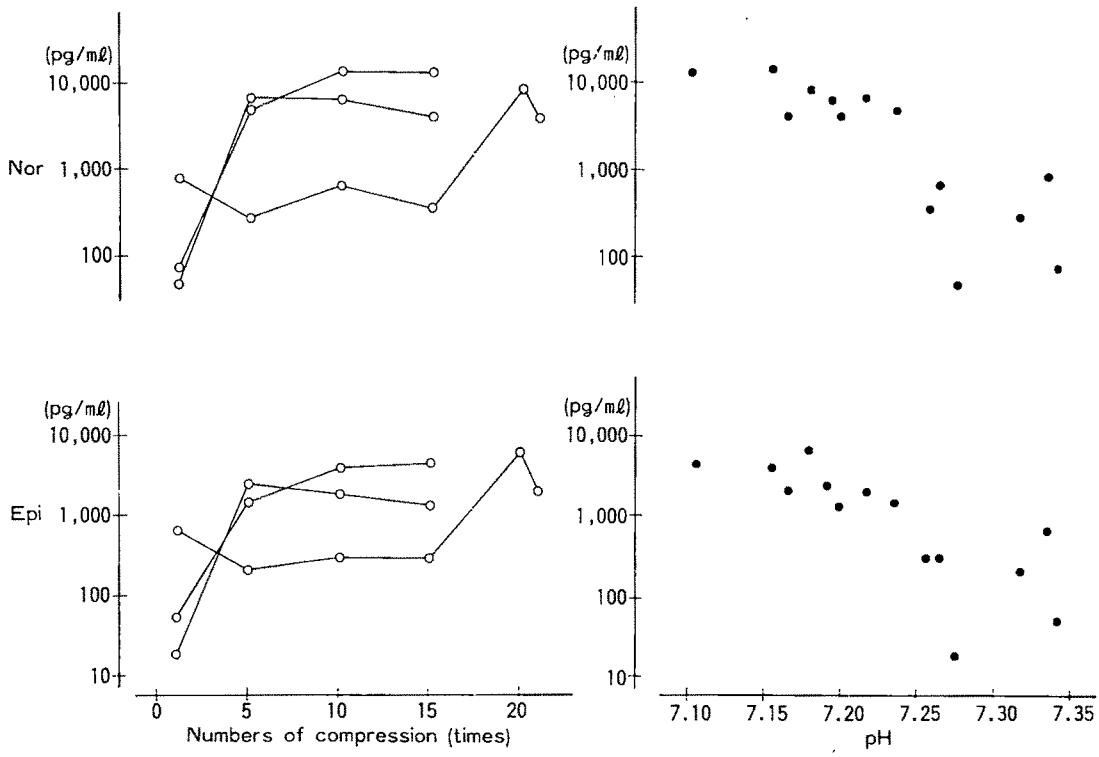


Fig. 4. Change in fetal plasma norepinephrine *(Nor)* and epinephrine *(Epi)* concentrations *(left)* and their relationships with fetal arterial pH *(right)*.

Table I. Correlation coefficients of T/QRS-1, T/QRS-2, and duration of elevated blood pressure during cord compression versus fetal arterial blood gas values and plasma catecholamine concentrations

	P_{O_2}	P_{CO_2}	*Base excess*	*pH*	*Log plasma epinephrine concentration*	*Log plasma norepinephrine concentration*	*Duration of elevated blood pressure during cord compression*
T/QRS-1	NS	0.7084	−0.7523	−0.8095	NS	0.7010	−0.8269
T/QRS-2	NS	0.7383	−0.6266	−0.7711	0.5445	0.7551	−0.8619
Duration of elevated blood pressure during cord compression	NS	−0.8569	0.7336	0.9024	NS	−0.6808	—

T/QRS-1, T/QRS just before compression. T/QRS-2, T/QRS 40 second after start of compression. $p < 0.01$. *NS*, Not statistically significant.

tions, and blood pressure during cord compression between two groups divided according to whether T/QRS-2 was >0.50. pH and base excess were higher, blood pressure during cord compression was longer, and P_{CO_2} and catecholamine concentrations were lower in the group in which T/QRS was <0.50 than in the group in which T/QRS was >0.50.

Comment

Variable decelerations in intrapartum FHR monitoring are thought to be caused by umbilical cord compression.[2, 3, 15] We previously observed variable decelerations in chronically instrumented lamb fetuses by compressing the umbilical cord repeatedly. In that experiment we found gradually progressing fetal acidemia, an increase in fetal catecholamine concentrations, and progress in fetal hypotension during cord compression, as the fetus became acidotic by repeated cord compression.[15] Regarding the progress in fetal acidemia seen in repetitive variable decelerations, Kubli et al.[4] stated that the fetus becomes acidotic when the nadir of variable decelerations is <70 beats/min or the duration of variable decelerations is >60 seconds. However, the depth or duration of variable decelerations does not always predict the pH of umbilical blood or neonatal Apgar score[5] in the clinical setting. Also in fetal sheep we observed only a weak correlation ($r = -0.337$) between arterial pH and depth of variable decelerations.[15]

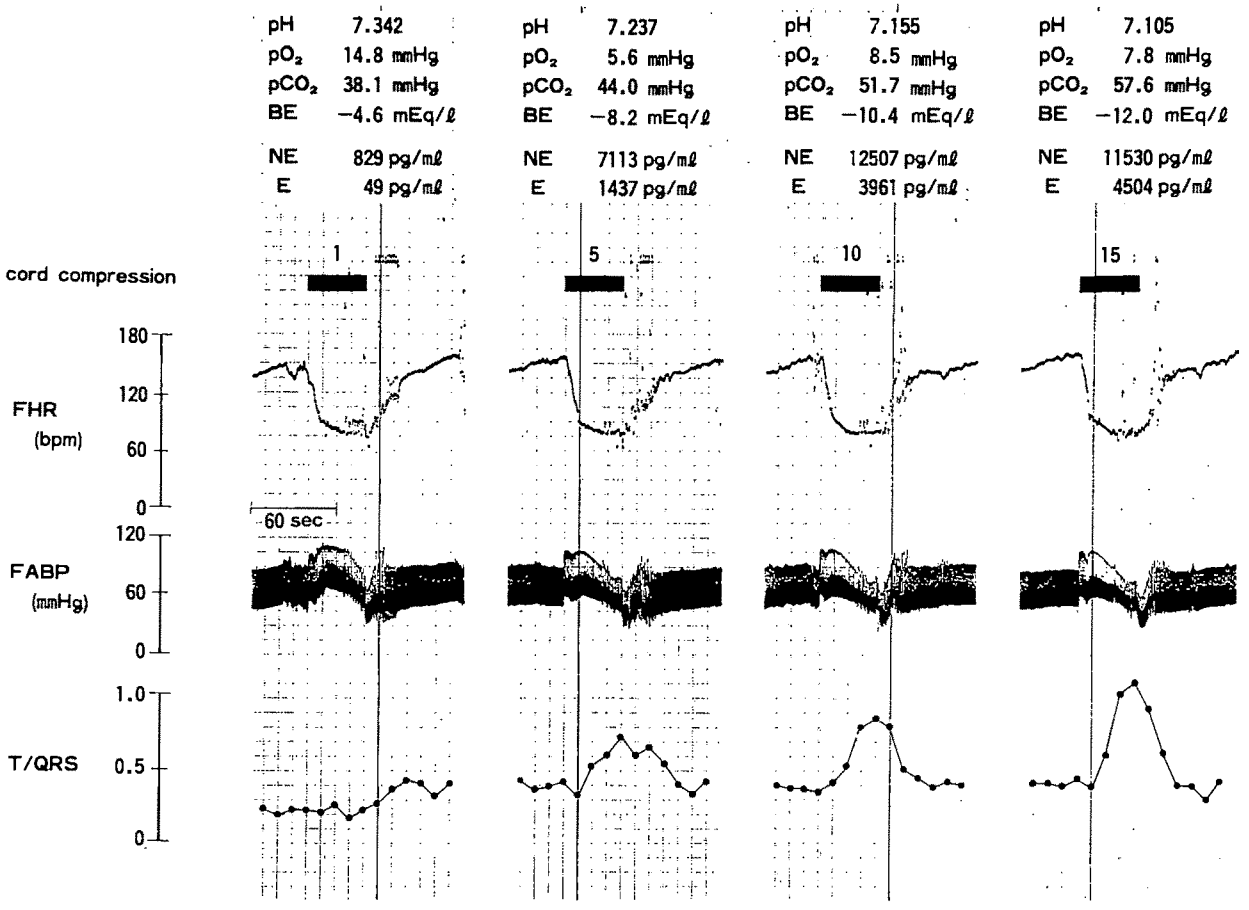


Fig. 5. Changes in FHR patterns, fetal arterial blood pressure *(FABP)*, T/QRS, fetal arterial gas values, and catecholamine concentrations with repeated umbilical cord compression. *BE*, Base excess; *NE*, norepinephrine; *Epi*, epinephrine.

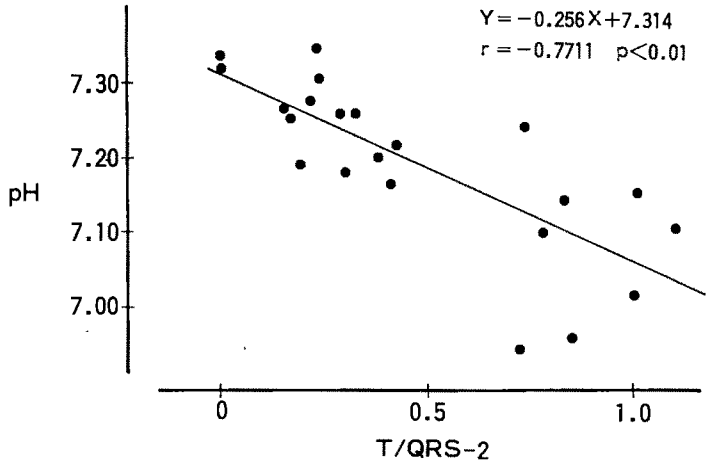


Fig. 6. Correlation between T/QRS during compression (T/QRS-2) and fetal arterial pH.

As a result, when repetitive variable decelerations are seen in FHR monitoring, the fetus may be acidotic, and cardiovascular function may be altered. Thus it is necessary to estimate the severity of fetal acidemia or altered cardiovascular function by evaluating parameters other than the depth or the duration of variable decelerations. In the current study we attempted to estimate the severity of fetal distress (progress in fetal

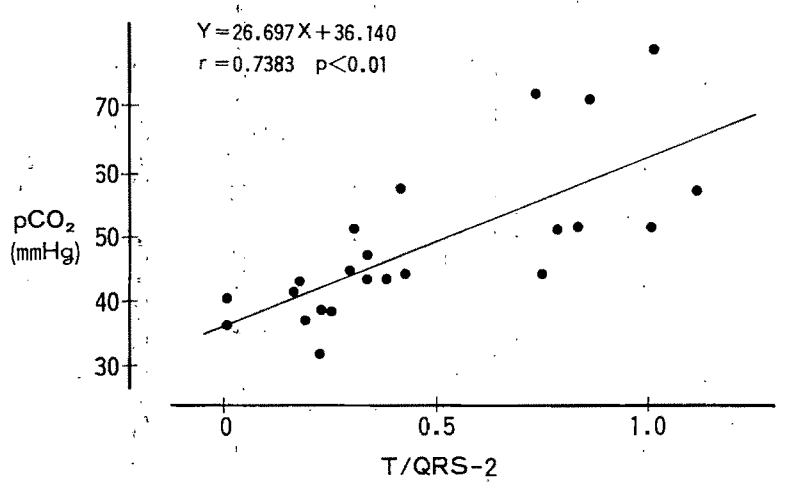


Fig. 7. Correlation between T/QRS during compression (T/QRS-2) and fetal arterial P_{CO_2}.

Table II. Sensitivity, specificity, and positive and negative predictive values of T/QRS in predicting fetal acidemia (pH <7.15)

	T/QRS-1 >0.30	*T/QRS-2 >0.50*
Sensitivity	100.0	100.0
Specificity	76.5	87.5
Positive predictive value	60.0	75.0
Negative predictive value	100.0	100.0

acidemia, increase in catecholamine concentrations and decrease in blood pressure during compression) by observing the change in T/QRS in fetal ECG when repetitive variable decelerations were produced by compressing the umbilical cord repeatedly in lamb fetuses.

T/QRS just before compression (T/QRS-1) correlates with fetal arterial pH, P_{CO_2}, base excess, catecholamine concentrations, and duration of elevated blood pressure during cord compression (Table I). T/QRS during compression (T/QRS-2) also correlates with fetal arterial pH (Fig. 6), P_{CO_2} (Fig. 7), catecholamine concentrations, and duration of elevated blood pressure during cord compression (Fig. 8). Thus we can estimate the severity of acidemia, catecholamine concentrations, and the decrease in fetal blood pressure during compression by observing T/QRS during and before the cord compression.

Several investigations into the change in T/QRS of fetal ECG during prolonged hypoxia have been performed in experimental animal studies. In mild hypoxia, change in T/QRS is thought to be mediated by activation of β-adrenergic receptors, because these changes in T/QRS are blocked by injection of the β-adrenergic receptor–blocking agent propranolol.[16] During severe hypoxia, change in T/QRS is considered to be a direct result of myocardial hypoxia, which causes metabolic acidosis with lactate accumulation.[9, 16] Rosen et al.[10] reported correlations between T/QRS and epinephrine concentrations and stated that an increase in T/QRS indicates an anaerobic switch in myocardial metabolism. Hoekegard et al.[8] found a relationship between T/QRS and myocardial glycogen by direct myocardial biopsy. In all, elevation of T/QRS by hypoxia indicates a negative energy balance in cardiac metabolism followed by anaerobic breakdown of glycogen.

With respect to periodic hypoxic stress, Widmark et al.[12] compressed the aorta of the ewe for 60 seconds and found that T/QRS increased significantly, without acidemia, because of the compression and returned to the precompression value after the release of compression. On the contrary, we found only a small change in T/QRS by umbilical cord compression when the fetus was not acidotic at the beginning of the experiment. However, as the fetus became acidotic, T/QRS during compression gradually increased with cord compression (Fig. 5). We speculate that without acidemia, elevated blood pressure persists during the compression period, maintaining coronary flow and compensating for the fall in arterial P_{O_2}, but when fetal acidemia develops, fetal blood pressure decreases during cord compression (Figs. 5 and 9), causing a decrease in coronary flow, which, together with the decrease in P_{O_2}, diminishes oxygen supply to the myocardium. These speculations are supported by the strong negative correlations ($r = -0.8619$, $p < 0.01$) between T/QRS-2 and duration of elevated blood pressure during cord compression in Fig. 8. Because duration of elevated blood pressure during cord compression correlated well ($r = -0.8569$, $p < 0.01$) with P_{CO_2} (Table I), we speculate that decrease in fetal blood pressure during cord compression is due to myocardial depression caused mainly by respiratory acidemia.

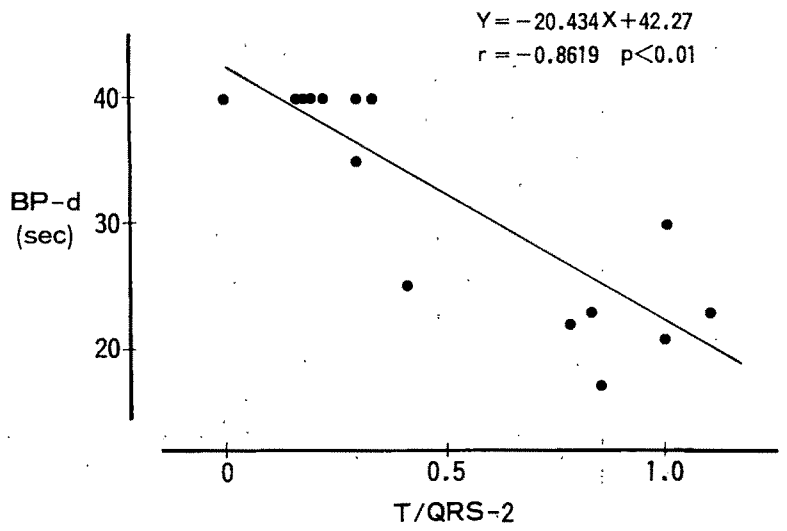


Fig. 8. Correlation between T/QRS during compression (T/QRS-2) and duration of elevated blood pressure *(BP-d)*.

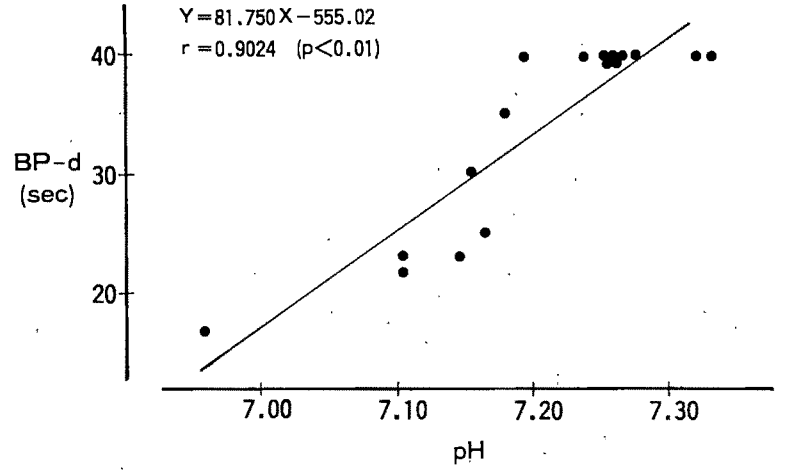


Fig. 9. Correlation between duration of elevated blood pressure *(BP-d)* and fetal arterial pH.

In clinical work several investigations with fetal scalp electrodes have been performed regarding the change in T/QRS during labor.[17-20] Lilja et al.[19] observed the fetal ECG with a microprocessor and reported that a T/QRS ratio <0.25 appeared to be a reliable sign of fetal well-being. Regarding the relationship between FHR pattern and the change in T/QRS, Pardi et al.[18] reported that an increased T-wave amplitude appeared in 15% of severe variable decelerations and in 48% of late decelerations; however, they did not show any definite relationship between T-wave height and fetal biochemical status. Further, there have been no observations reported relating to T/QRS criteria during FHR deceleration by which one can predict fetal acidemia when decelerations appear in FHR monitoring.

For the purpose of predicting fetal acidemia (pH < 7.15), we calculate the sensitivity, specificity, and predictive values of T/QRS (both T/QRS-1 and T/QRS-2) shown in Table II. T/QRS-1 and T/QRS-2 values of 0.30 and 0.50, respectively, are reliable criteria for predicting fetal acidemia.

T/QRS-2 correlated well with catecholamine concentrations, as shown in Table I; therefore we can estimate fetal catecholamine concentrations by measuring T/QRS during decelerations. Fetal catecholamines are essential in redistributing cardiac output to vital organs when hypoxia occurs. Lorijn and Longo[21] reported that in fetal lambs redistribution to vital organs occurs when norepinephrine concentrations are about 4500 pg/ml by continuous infusion. Table III shows the difference in pH, catecholamine concentrations, and duration of elevated blood pressure during cord compression between two groups divided according to whether the T/QRS values were >0.50. If T/QRS-2 is <0.50, the fetus is not so acidotic, catecholamines can be se-

Table III. Classification of T/QRS during cord compression into two groups

	T/QRS during cord compression		
	<0.50 (0.245 ± 0.129)	*>0.50 (0.891 ± 0.137)*	*p Value*
pH	7.255 ± 0.055	7.084 ± 0.102	<0.005
Po_2 (mm Hg)	16.0 ± 7.0	14.5 ± 6.6	NS
Pco_2 (mm Hg)	42.6 ± 6.4	59.8 ± 12.6	<0.005
Base excess (mEq/L)	−8.0 ± 2.5	−12.7 ± 3.2	<0.005
Plasma norepinephrine (pg/ml)	4488 ± 3296	10383 ± 2874	<0.005
Plasma epinephrine (pg/ml)	1411 ± 1849	3301 ± 1637	NS
Duration of elevated blood pressure during cord compression (sec)	38.2 ± 4.6	25.1 ± 7.6	<0.005

Data are mean ± SD. *NS,* Not statistically significant.

creted further, and elevated blood pressure is maintained. However, when T/QRS-2 is >0.50, the fetus becomes acidotic and cannot further secrete catecholamines and fetal blood pressure decreases during deceleration. As shown in Fig. 5, the change in T/QRS is just a mirror image of FHR deceleration. Thus T/QRS-2 appears to be almost identical to T/QRS near the bottom of the FHR deceleration. This means that if a T/QRS >0.50 is observed near the bottom of the variable deceleration, fetal reserve to tolerate cord compression appears to be limited, and some kind of intervention may be necessary to prevent fetal compromise.

During human labor we usually obtain the fetal ECG signal by fetal scalp electrode. In that system the sensitivity vector is not parallel to the fetal longitudinal axis. In the current study we used a fetal unipolar limb lead that is similar to aVF, which may be nearly parallel to the fetal longitudinal axis. Thus we cannot apply the findings of this study directly to fetal ECG performed conventionally during labor. A sensitivity vector that is parallel to the fetal vertical axis could be performed by placing electrodes on the fetal scalp and maternal thigh, as Lindecrantz and Widmark[12] have suggested. In that lead ECG waveforms would be similar to that of fetal aVF, and we could apply the findings of the current study to human fetus during labor.

As for changes other than T-wave amplitude in the fetal ECG induced by hypoxia, there are some clinical observations regarding P wave or PQ interval.[17,18] However, there are few observations of these in animal experiments. By umbilical cord compression, change in P wave and PQ interval might occur as a result of vagal stimulation or direct hypoxic effect or catecholamine release. We do not discuss here, however, whether valuable observations might be made of changes in P wave or PQ intervals.

In the current study we examined decelerations caused by repeated cord compression. Further investigations are needed to apply these findings to the case of late decelerations, because there are differences between late and variable decelerations, such as the absence of a sudden increase in fetal blood pressure during deceleration in late decelerations.

In conclusion, when variable decelerations are seen in intrapartum FHR monitoring, fetal condition can be predicted according to whether T/QRS at the bottom of the deceleration is >0.50. Further clinical investigations are needed to apply these findings to intrapartum management.

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Changes in human platelet angiotensin II binding sites associated with early pregnancy

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We have demonstrated that in very early pregnancy the elevated levels of the components of the renin-angiotensin system are associated with a significant diminution in platelet angiotensin II binding ($p < 0.01$). (AM J OBSTET GYNECOL 1992;166:255-6.)

Key words: Angiotensin II, pregnancy, platelet membrane glycoproteins, renin-angiotensin system

A cross-sectional study has suggested that the level of human platelet angiotensin II binding sites in pregnancy parallels the diminution in pressor responsiveness to infused angiotensin II, with platelet angiotensin II binding being significantly lower in patients in the first trimester than in nonpregnant women.[1] The purpose of this, the first longitudinal study of platelet angiotensin II binding in pregnancy, was to study further platelet angiotensin II binding levels in early pregnancy.

Thirty nulliparous, normotensive women were recruited for the study, after having been seen by their general practitioners for advice about preconception health care. No subject had been using hormonal contraception for at least 6 months, and all had regular menstrual cycles. All subjects were investigated between days 5 and 9 of the menstrual cycle, because an effect of the menstrual cycle on platelet angiotensin II binding has been demonstrated.[2] Approximately 20 ml of blood was taken from each subject for determination of platelet angiotensin II binding, as previously described.[1] Measurements of plasma angiotensin II, renin substrate, and renin concentration were also performed.

Twelve women (aged 24 to 33 years, median 27) conceived within 1 year of the initial investigation. A blood sample was obtained between weeks 5 and 8 after the last menstrual period, and further samples were obtained at 11 and 18 weeks' gestation.

A significant effect of early pregnancy on platelet angiotensin II binding was found ($p < 0.01$, Friedman

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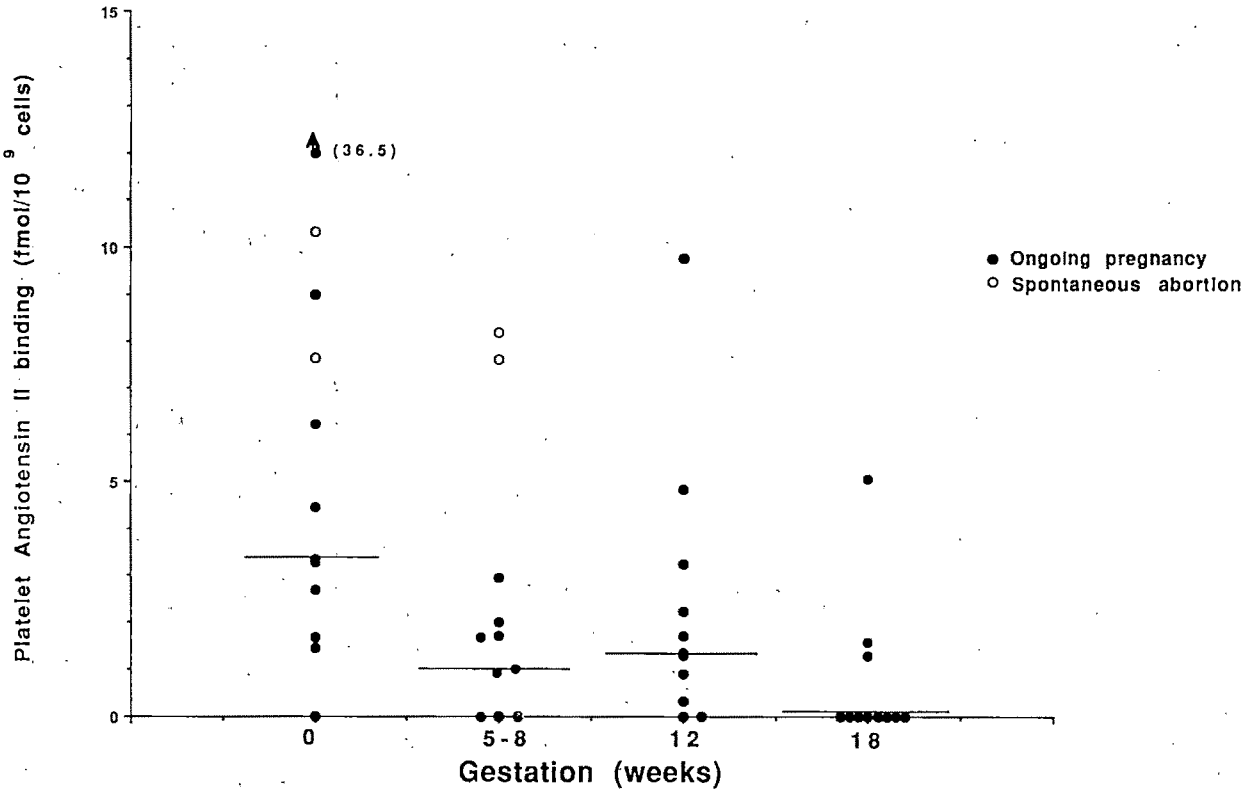


Fig. 1. Human platelet angiotensin II binding in early pregnancy.

analysis of variance) (Fig. 1) Platelet angiotensin II binding at 5 to 8 weeks' gestation (median value 1.2 fmol/10^9 cells) was significantly lower than when measured before conception (3.4 fmol/10^9 cells), and the level at 18 weeks' gestation (0.0 fmol/10^9 cells) was significantly lower than that at 11 weeks' gestation (1.5 fmol/10^9 cells) (in each case $p = 0.02$, Wilcoxon matched-pairs, signed-ranks test).

Plasma angiotensin II levels at 5 to 8 weeks' gestation (median 40.3 pg/ml, range 14.8 to 59.2) were significantly higher than those before conception (12.1 pg/ml, 2.8 to 26.3, $p < 0.02$, Wilcoxon matched-pairs, signed-ranks test), although there were no subsequent significant changes in plasma angiotensin II level. The plasma renin substrate level and plasma renin concentration also rose in early pregnancy, demonstrating a significant effect on both the hormones (in each case $p < 0.01$, Friedman analysis of variance).

Two further women conceived but had spontaneous abortions at 8 and 10 weeks. At 5 to 8 weeks' gestation platelet angiotensin II binding was not reduced (Fig. 1), and the values of plasma angiotensin II were below the median value of the other 12 women.

The diminution in angiotensin II binding to platelets (a suggested model of vascular smooth muscle[1]), which accompanied the elevated levels of the components of the renin-angiotensin system and was demonstrable as early as 5 to 8 weeks' gestation, is thus a very early adaptation to pregnancy. The changes found may represent a continuation of the low binding found in the luteal phase of the menstrual cycle,[2] the return to follicular levels not occurring if the woman conceives.

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The maternal and fetal effects of milrinone and dopamine in normotensive pregnant ewes

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The safety of milrinone, administered during gestation, was evaluated in seven chronically instrumented pregnant ewes and their fetuses. Each of the following six intravenous regimens was administered in random order: milrinone, 75 $\mu g \cdot kg^{-1}$; over 1 minute, followed by 240-minute infusions of the drug at a rate of 1, 2, or 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$; dopamine 5 or 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ over 60 minutes; or normal saline solution, 0.5 $ml \cdot min^{-1}$ for 240 minutes. Maternal and fetal acid-base parameters, heart rate, and blood pressure were monitored as were the ewe's venous and intraamniotic pressures and uterine blood flow. Milrinone concentrations determined in maternal arterial blood samples obtained during 1 and 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusions were found to be within the human therapeutic range. Bolus injection of milrinone, as well as the lowest drug infusion, resulted in no significant changes in uterine blood flow, whereas 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ milrinone infusion led to a 14% to 19% increase in uterine blood flow between 120 and 240 minutes. With the highest milrinone infusion, this increase was approximately 20%. In contrast, with both dopamine infusions, a dose-relatec decrease in uterine blood flow of 15% to 26% occurred between 15 and 60 minutes. No milrinone could be detected in any fetal plasma samples. Fetal arterial pH and blood gas tensions did not change during milrinone infusions. Dopamine 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ led to a progressive decrease in fetal arterial pH and an increase in $Paco_2$, which may have been related to similar changes in the ewe. It is concluded that milrinone has no adverse effects on uterine blood flow and fetal well-being when administered during ovine pregnancy. (Am J Obstet Gynecol 1992;166:257-62.)

Key words: Cardiotonic drugs, milrinone, dopamine; effects: maternal and fetal; pregnancy, sheep

Vasopressor and inotropic agents, which exert their effects by adrenergic receptor stimulation, may improve maternal cardiovascular status but, at the same time, reduce uteroplacental perfusion.[1-5] Recently formulated bipyridine compounds, amrinone and milrinone, are potent cardiotonic drugs having a different mechanism of action, that is, inhibition of myocardial phosphodiesterase activity.[6] These drugs also have a mild direct pulmonary and systemic vasodilator effect.[6, 7] Little is known of their efficacy and safety during pregnancy. Amrinone has been reported to have no adverse effect on uterine blood flow in the pregnant baboon, but no data regarding plasma concentrations, placental transfer, and/or fetal effects of the drug were presented.[8] Milrinone, a newer agent, is a methyl carbonitril derivative of amrinone.[9] In animal and human tests, its use has resulted in enhanced cardiac performance.[6, 7, 9] Potential advantages of milrinone over amrinone are that it can be formulated in an oral preparation, as well as a parenteral one, and it is less likely to cause side effects, such as gastrointestinal disturbances or thrombocytopenia.[6, 9] To date there have been no reports on the safety of milrinone administered during pregnancy. The current study was undertaken to evaluate the maternal and fetal effects of milrinone in chronically instrumented pregnant sheep.

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Material and methods

Pregnant sheep near term of gestation, term being 148 days, were studied according to a protocol approved by the Institutional Animal Care and Use Committee. Details of surgical preparation have been described previously.[10] Briefly, after an overnight fast, ewes were given a spinal anesthetic, tetracaine hydrochloride, 12 to 14 mg, supplemented by a dilute thiopental sodium drip as needed. The maternal abdominal aorta and common iliac vein were cannulated with poly-

ethylene catheters via the femoral vessels. Laparotomy and hysterotomy were then performed for cannulation of the fetal common carotid artery and jugular vein. A catheter was also placed in the amniotic cavity. A 4 mm "R" series pulse transit-time ultrasonographic flow probe (Transonic Systems, Inc., Ithaca, N.Y.), sensitive enough to detect changes in blood flow of at least 10%, was secured around a branch of the uterine artery supplying the pregnant horn. During abdominal closure, all catheters and the flowmeter cable were tunneled subcutaneously and exteriorized within a pouch attached to the ewe's flank. Finally, after local infiltration with 2-chloroprocaine, a 16-gauge venous cannula was inserted percutaneously into the ewe's jugular vein.

Animals were then allowed to recover for at least 4 days, the period of time necessary for flow probe stabilization. Antibiotics were administered twice daily to the mother and fetus throughout the recovery and study period.

On the day of experiment, the ewe was weighed and kept standing in a cart. During a control period of at least 60 minutes and throughout the study, maternal and fetal heart rate and arterial blood pressure were continuously recorded on a polygraph (Siemens Corp., Iselin, N.J.). The ewe's venous pressure (common iliac vein) and intraamniotic pressure were also recorded, as was uterine blood flow. Fetal arterial blood pressure was corrected for changes in intraamniotic pressure.

On separate days, the ewe was given one of the following six regimens in random order: (1) an intravenous injection (jugular catheter) of milrinone 75 $\mu g \cdot kg^{-1}$ over 1 minute, followed by a 240-minute infusion of the drug at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$; (2) same milrinone bolus injection followed by a 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion; (3) same milrinone bolus injection followed by a 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion; (4) dopamine 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion over 60 minutes; (5) dopamine 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion, also over 60 minutes; (6) normal saline solution infused at 0.5 $ml \cdot min^{-1}$ over 240 minutes.

Only a saline solution infusion could be followed by a drug study on the same day but a drug infusion required a recovery period of 1 day. In preliminary studies bolus injections of milrinone 75 $\mu g \cdot kg^{-1}$ followed by 1 or 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusions of the drug were found to result in plasma drug concentrations of 80 to 400 $ng \cdot ml^{-1}$, which correspond to therapeutic levels in human subjects.[9] Dopamine, known to reduce uterine perfusion in a dose-dependent manner,[4] was administered at infusion rates that resulted in a 15% to 25% decrease in uterine artery blood flow in our preliminary studies. The duration of dopamine infusions was limited to 60 minutes to preserve fetal viability.

Arterial blood samples were drawn from the ewe and fetus during the control period and at the end of bolus injection of milrinone. During infusions of milrinone or saline solution, samples were obtained at 5, 15, 30, and 60 minutes and hourly thereafter, up to 240 min. During dopamine administration samples were taken at 15, 30, and 60 minutes. The volume of each fetal blood sample was 1.25 ml in the milrinone 1 and 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 0.25 ml in all other studies. All samples were analyzed for pH and gas tensions with standard microelectrodes. Aliquots obtained from the 1 and 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ milrinone studies were centrifuged, and plasma was separated and frozen at $-20°$ C to be subsequently analyzed in the laboratories of the Sterling Research Group for milrinone concentrations with high-pressure liquid chromatography with ultraviolet detection. The limit of sensitivity was 5 $ng \cdot ml^{-1}$, and the coefficient of variation was 3.7% ± 2.5% at 120 $ng \cdot ml^{-1}$.

Uterine vascular resistance was calculated with the standard formula:

$$UVR = \frac{MABP - CVP}{UBF}$$

where UVR is uterine vascular resistance in dynes · sec · cm^{-5}, UBF is uterine blood flow in $ml \cdot min^{-1}$, MABP is mean maternal arterial blood pressure, and CVP is central venous pressure, both in millimeters of mercury.[5]

Changes in monitored and measured variables were evaluated for statistical significance with analysis of variance for repeated measures (Sheffé F test). A p value <0.05 was considered significant. All results are expressed as mean ± SE.

Results

Seven ewes at gestational ages ranging from 121 to 138 days were studied. The number of experiments performed for each infusion regimen was as follows: milrinone 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (milrinone 1 μg), $n = 6$; milrinone 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (milrinone 2 μg), $n = 8$; milrinone 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$, (milrinone 4 μg), $n = 7$; dopamine 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (dopamine 5 μg), $n = 6$; dopamine 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (dopamine 10 μg), $n = 6$; and saline solution, $n = 5$. A saline solution study was followed by a milrinone study in three instances and by dopamine in the remaining two.

Ewes. The mean peak milrinone plasma concentration of 644 ± 45 $ng \cdot ml^{-1}$ occurred at the end of bolus injection (Fig. 1). After 60 minutes of milrinone 1 μg and milrinone 2 μg infusions, the plasma concentration declined to 117 ± 10 and 171 ± 9 $ng \cdot ml^{-1}$, respectively, and remained essentially unchanged thereafter.

The bolus injection of milrinone resulted in an increase in heart rate of approximately 50% (Table I). This tachycardia reverted to a normal heart rate after 30 minutes of milrinone 1 μg infusion but persisted for

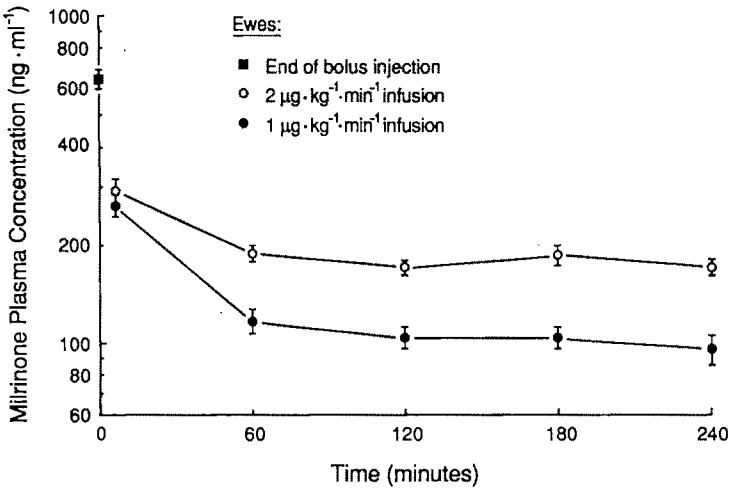


Fig. 1. Maternal plasma concentrations of milrinone (ng · ml^{-1}, mean ± SE) after bolus injection of 75 μg · kg^{-1} (time 0) and at 5, 60, 120, 180, and 240 minutes of infusion.

Table I. Values for maternal heart rate (beats per minute, mean ± SE)

Time (min)	*Saline solution*	*Milrinone 1 μg*	*Milrinone 2 μg*	*Milrinone 4 μg*	*Dopamine 5 μg*	*Dopamine 10 μg*
0	108 ± 4	109 ± 6	102 ± 3	98 ± 4	110 ± 3	100 ± 2
End of bolus	103 ± 2	158 ± 7*	137 ± 8*	147 ± 9*		
5	100 ± 2	163 ± 3*	153 ± 9*	166 ± 5*		
15	104 ± 4	160 ± 3*	152 ± 7*	160 ± 8*	121 ± 2	105 ± 5
30	103 ± 2	146 ± 5*	145 ± 7*	147 ± 11*	118 ± 6	117 ± 7*
60	100 ± 2	128 ± 3	138 ± 5*	157 ± 9*	117 ± 6	120 ± 6*
120	100 ± 2	118 ± 3	131 ± 6*	152 ± 11*		
180	97 ± 3	117 ± 3	125 ± 4	144 ± 7*		
240	97 ± 1	116 ± 3	126 ± 5	143 ± 7*		

*Significantly different from control ($p < 0.05$).

up to 120 and 240 minutes in ewes given the milrinone 2 μg and milrinone 4 μg infusions, respectively. Dopamine 10 μg led to an approximately 20% increase in heart rate after 30 minutes of infusion, which was sustained up to the end of the study. No significant changes in heart rate were noted with the dopamine 5 μg infusion.

Milrinone 1 μg infusion did not result in any change in maternal blood pressure, whereas at 15 and 30 minutes of milrinone 2 μg infusion, there was a small but statistically significant reduction in mean arterial blood pressure from a control value of 93 ± 4 to 85 ± 3 mm Hg. A similar 10% decrease in mean arterial blood pressure at 15, 30 and 60 minutes seen with the highest milrinone infusion rate failed to reach statistical significance. No statistically significant changes in mean arterial pressure were noted with either dopamine infusion.

Intraamniotic and central venous pressures remained unchanged throughout all experiments. Control uterine blood flows were similar among groups; for example, saline solution, 356 ± 81 ml · min^{-1}; milrinone 1 μg, 344 ± 52 ml · min^{-1}; milrinone 2 μg, 307 ± 47 ml · min^{-1}; milrinone 4 μg, 312 ± 59 ml · min^{-1}; dopamine 5 μg, 396 ± 69 ml · min^{-1}; and dopamine 10 μg, 390 ± 67 ml · min^{-1}. Bolus injection of milrinone, as well as milrinone 1 μg infusion, resulted in no significant changes in uterine blood flow (Fig. 2). Milrinone 2 μg infusion led to increases in uterine blood flow of 13.9% ± 3.1%, 16.8% ± 3.8%, and 18.6% ± 3.4%, at 120, 180, and 240 minutes, respectively. With milrinone 4 μg infusion there was an 18.9% ± 7.4% increase in uterine blood flow at 180 minutes and a 22.3% ± 7.5% increase at 240 minutes. In contrast, dopamine administration caused a dose-related decrease in uterine blood flow between 15 and 60 minutes. At 60 minutes uterine blood flow was 15.0% ± 2.0% with dopamine 5 μg and 26.3% ± 6.5% with dopamine 10 μg (Fig. 2). Neither the bolus nor milrinone 1 μg infusion altered uterine vascular resistance, but milrinone 2 μg infusion resulted in a reduction in uterine vascular resistance ranging from 15% to 17% (Fig. 3). Changes in uterine vascular resistance seen with milrinone 4 μg infusion failed to

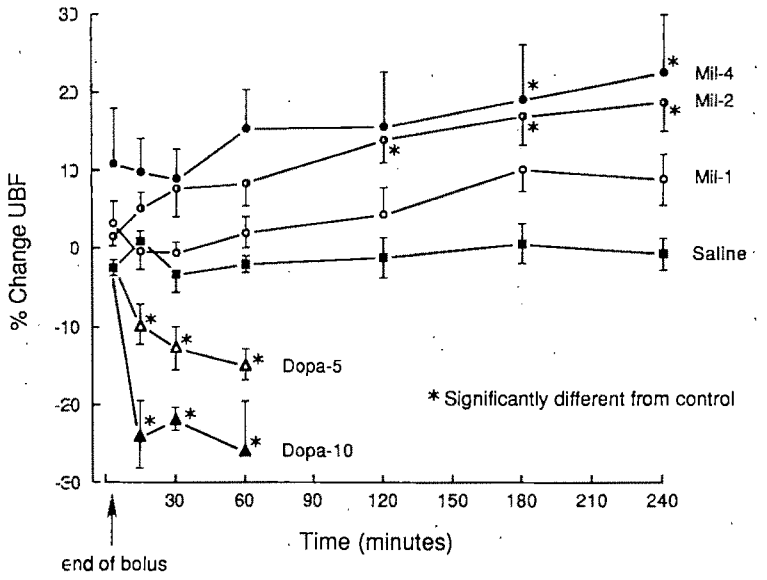


Fig. 2. Percent change (mean ± SE) in uterine blood flow *(UBF)* after bolus injection of 75 μg · kg^{-1} milrinone over 1 minute, at 15, 30, 60, 120, 180 and 240 minutes of milrinone infusion, and at 15, 30, and 60 minutes of dopamine infusion. *Mil-1,* Milrinone 1 μg; *Mil-2,* milrinone 2 μg; *Mil-4,* milrinone 4 μg; *Dopa-5,* dopamine 5 μg; *Dopa-10,* dopamine 10 μg.

reach statistical significance. A 25% to 40% increase in uterine vascular resistance was noted during dopamine 10 μg infusion.

Infusions of saline solution or milrinone did not affect maternal arterial pH or blood gas tensions at any time. In contrast, dopamine reduced maternal arterial pH at 15 and 60 minutes (Table II). Changes in arterial Po_2 were only significant with dopamine 5 μg at 15 minutes.

Fetuses. Milrinone could not be detected in any fetal plasma samples. Heart rate and mean arterial blood pressure were unaffected by infusions of saline solution, milrinone, or dopamine. Before infusion these values were: saline solution (n = 4), 166 ± 12 beats · min^{-1} and 46 ± 1 mm Hg; milrinone 1 μg (n = 6), 171 ± 6 beats · min^{-1} and 44 ± 2 mm Hg; milrinone 2 μg (n = 7), 165 ± 9 beats · min^{-1} and 42 ± 2 mm Hg; milrinone 4 μg (n = 6), 170 ± 6 beats · min^{-1} and 43 ± 2 mm Hg; dopamine 5 μg (n = 4), 156 ± 7 beats · min^{-1} and 42 ± 2 mm Hg; dopamine 10 μg (n = 4), 160 ± 11 beats · min^{-1} and 45 ± 5 mm Hg, respectively. Because of technical problems with the polygraph these variables could not be recorded in all experiments. Arterial blood pH and gas tensions, however, were determined in all fetuses and were found to be unaffected by infusions of saline solution, milrinone, and dopamine 5 μg. With dopamine 10 μg there was a progressive decrease in fetal arterial pH from 7.36 ± 0.02 to 7.30 ± 0.01 at 60 minutes and an increase in Pco_2 from 45 ± 3 to 50 ± 3 mm Hg at 15 minutes and 53 ± 3 mm Hg at 60 minutes (Table II).

Comment

These data suggest that milrinone produces no adverse effects in the pregnant ewe or fetus. At plasma concentrations considered to be therapeutic in humans, namely, 80 to 400 ng · ml^{-1},[9] the drug did not reduce uterine blood flow in pregnant ewes. On the contrary, at the two higher dosages, a small increase in uterine blood flow could be demonstrated after 2 to 4 hours of drug infusion. However, a potential limitation of measuring uterine artery blood flow is that it does not provide data on the relative placental and nonplacental perfusion.[11] Myometrial blood flow may be increased at the expense of placental perfusion by vasodilating agents, such as isoproterenol.[12] Further, the calculation of uterine vascular resistance with the use of values from a single vessel may not accurately reflect the vascular resistance throughout the uterus.

The uterine vascular bed is generally regarded to be maximally dilated at term of ovine pregnancy.[13] However, small increases in uterine blood flow have been found to occur in nonlaboring pregnant sheep given an agent with mild vasodilator activity, such as halothane.[14] Milrinone is also considered to be a mild vasodilator,[6, 7] and in the current study milrinone 2 μg infusion resulted in a decrease in uterine vascular resistance. Milrinone 4 μg infusion led to an increase in uterine blood flow of a magnitude similar to that of the intermediate dose but without the decrease in uterine vascular resistance achieving statistical significance. This was most probably due to insufficient power of statistical analysis (32%), because uterine vascular resistance could not be calculated in all milrinone 4 μg

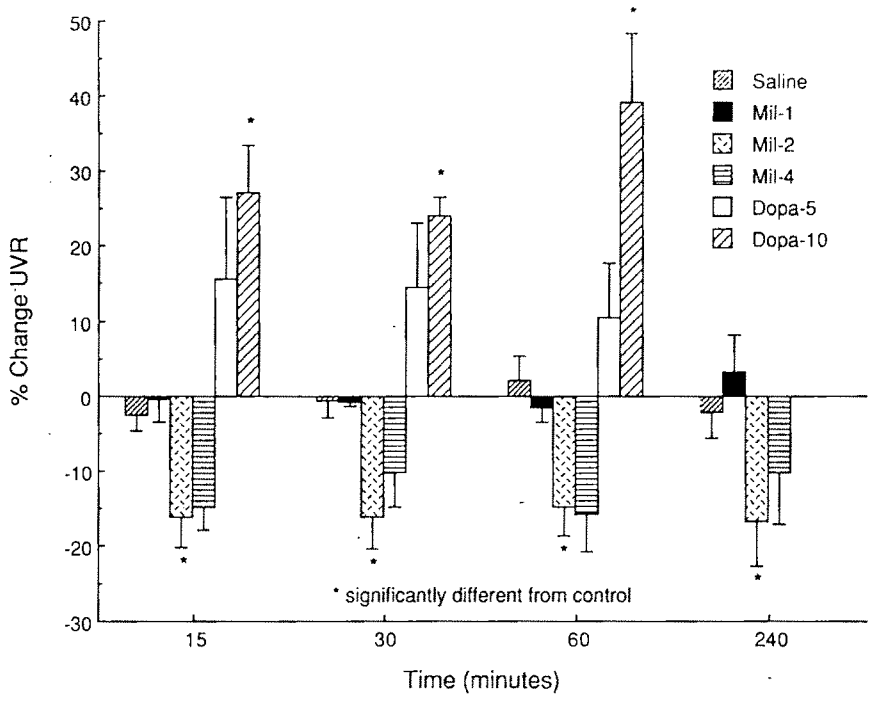


Fig. 3. Percent change (mean ± SE) in uterine vascular resistance *(UVR)* after 15, 30, 60, and 240 minutes of milrinone and 15, 30, and 60 minutes of dopamine infusion. *Mil-1,* Milrinone 1 μg; *Mil-2,* milrinone 2 μg; *Mil-4,* milrinone 4 μg; *Dopa-5,* dopamine 5 μg; *Dopa-10,* dopamine 10 μg.

studies. Furthermore, there also appears to be a ceiling to the cardiovascular effects of milrinone,[15] which may have been reached with milrinone 2 μg infusion. At the highest milrinone dose, drug-induced changes in uterine vascular resistance and uterine blood flow may have also been limited by maternal tachycardia and, possibly, reduced ventricular filling.

In contrast to milrinone, dopamine administration at two infusion rates used in clinical practice resulted in a dose-related decrease in uterine blood flow. Other investigators have also found dopamine to reduce uterine blood flow in normotensive and hypotensive pregnant ewes.[4, 5] This has been attributed to an increase in uterine vascular resistance, particularly at higher drug dosages.[5, 16] In this study dopamine 10 μg increased uterine vascular resistance by 45%. The reason for a reduction in uterine blood flow with dopamine 5 μg infusion is less clear because the increase in uterine vascular resistance did not achieve statistical significance. This could be related to the observation made by others that dopamine at lower dosages does not greatly affect uterine vascular resistance in sheep.[16] An increase in uterine tone caused by dopamine, which in itself may reduce uterine blood flow,[16] was not seen in any of our experiments and may be explained by the lower infusion rates of the drug used in our study.

An intravenous bolus injection of milrinone 75 $\mu g \cdot kg^{-1}$ resulted in significant maternal tachycardia. However, one should note that plasma concentrations of the drug after bolus administrations were well above the recommended therapeutic range.[9] High doses of milrinone have been shown to cause tachycardia with no additional improvement in hemodynamic status.[9, 17] Dopamine 10 μg infusion, which is an appropriate dose for clinical use, also resulted in moderate maternal tachycardia.

Table II. Values (mean ± SE) for maternal and fetal arterial pH and blood gas tensions (millimeters of mercury) before and after dopamine infusion

	0 min	*15 min*	*60 min*
Dopamine 5 μg			
Mothers			
pH	7.54 ± 0.02	7.48 ± 0.01*	7.46 ± 0.02*
P_{CO_2}	35 ± 2	37 ± 2	38 ± 2
P_{O_2}	96 ± 3	80 ± 2*	82 ± 6
Fetuses			
pH	7.35 ± 0.02	7.34 ± 0.02	7.33 ± 0.01
P_{CO_2}	44 ± 3	46 ± 2	47 ± 3
P_{O_2}	28 ± 1	27 ± 2	26 ± 2
Dopamine 10 μg			
Mothers			
pH	7.53 ± 0.01	7.46 ± 0.01*	7.46 ± 0.01*
P_{CO_2}	36 ± 1	40 ± 2	40 ± 2
P_{O_2}	92 ± 3	82 ± 1	90 ± 3
Fetuses			
pH	7.36 ± 0.02	7.33 ± 0.01	7.30 ± 0.01*
P_{CO_2}	45 ± 3	50 ± 3*	53 ± 3*
P_{O_2}	25 ± 2	26 ± 2	26 ± 2

*Significantly different from control ($p < 0.05$).

Maternal arterial pH and blood gas tensions were unchanged during infusion of saline solution or milrinone, whereas administration of dopamine resulted in a significant reduction in arterial pH and a trend toward an increase in $PaCO_2$. It has been proposed that in sheep dopamine may cause pulmonary vasoconstriction and an increase in dead space ventilation.[4, 5, 18] A small decrease in PaO_2 has also been noted in these studies, but in the current one this occurred only in the dopamine 5 μg experiment.

The rate of milrinone 1 μg infusion was approximately 30% greater than the maximum recommended dose for human use.[9] Nonetheless, it resulted in plasma drug concentrations corresponding to the lower limit of the therapeutic range in man. The greater drug requirement in pregnant sheep may be related not only to species differences but also to gestational changes in blood volume, its constituents, and cardiac output.[19-22] Pregnancy has been shown to alter the pharmacokinetics of several drugs.[23] We are unaware of any reports on the effects of pregnancy on milrinone disposition.

It is of interest that even with the use of a very sensitive analytic technique, milrinone could not be detected in fetal plasma at any time during the study. This could be due to a slow rate of placental diffusion and possibly to placental degradation of milrinone, which has an amine structure.[8] The ovine placenta is syndesmochorial while that in humans is hemochorial; therefore the lack of placental transmission might not apply to pregnant women.

Dopamine did not affect fetal heart rate or mean arterial blood pressure. However, with dopamine 10 μg infusion the fetus demonstrated significant arterial acidemia. It would be tempting to assume that this was related to the approximately 25% reduction in uterine blood flow observed with dopamine infusion, but others have shown that ovine uterine blood flow may be decreased by as much as 50% without causing fetal asphyxia.[13] Thus maternal acid-base derangement was the most likely cause of fetal acidemia noted in the dopamine 10 μg experiments.

In conclusion, our data suggest that milrinone does not have adverse effects on uterine blood flow and fetal well-being when administered to pregnant sheep. The clinical relevance of these data is limited by the fact that they were obtained in healthy, normotensive animals and thus may not apply to pregnant women with impaired cardiovascular performance. Obviously, careful clinical trials of milrinone would first be required to ascertain its safety and efficacy during human pregnancy.

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LETTERS TO THE EDITORS

Periconceptional exposure to topical 5-fluorouracil

To the Editors: The recent article by Odom et al. (Odom LD, Plouffe L Jr, Butler WJ. 5-Fluorouracil exposure during the period of conception: report on two cases. AM J OBSTET GYNECOL 1990;163:76-7) calls to attention the theory that periconceptional exposure to topical vaginal 5-fluorouracil (5-FU) may be on the rise and speculates about two successful pregnancies exposed to 5-FU.

During 1990 we counseled seven patients referred for risk assessment because of periconceptional exposure to intravaginal topical 5-FU during treatment of human papillomavirus lesions. The patients were informed that, although it is suspected that 5-FU is a teratogenic agent, there is no information available about its vaginal topical use. However, on the basis of the all-or-none response we thought that the situation probably did not pose a significant risk. Of these patients, five have already been delivered of their infants. Four infants were normal; one 26-year-old woman gave birth to a female infant with Down syndrome (47,XX,+21 karyotype). Studies to determine the parental origin and meiosis stage of the nondisjunction event were not successful.

It is known that teratogenic effects have been produced with 5-FU in all animal species tested[1] and that there is evidence to suggest teratogenicity in humans. A different hypothesis could explain a potential association between periconceptional exposure to an antineoplastic and occurrence of chromosomal abnormalities.[2-4] It seems appropriate to be very cautious about speculating as to possible conclusions. Thus it does not seem that two negative cases (no teratogenic effects) can support the final statement of the article by Odom et al.: "It is likely that an uneventful ongoing pregnancy after periconceptional exposure will not display any adverse effects." If, for instance, periconceptional exposure of 5-FU would increase the incidence of birth defects three times from, say, 3% to 9%, it would be necessary to monitor at least 85 cases to detect this hypothetical increment with 95% confidence. A teratogenic effect could exert its action not by increasing the overall rate of birth defects but by producing a selective increment of birth defects (remember the thalidomide and diethylstilbestrol experiences).[5, 6] Furthermore, recent studies have suggested that to appeal to the concept of all-or-none effect could be an oversimplification.[7]

Thus much more information is necessary to assess potential risks of human developmental toxicity associated with the use of vaginal topical 5-FU.

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Reply

To the Editors: We appreciate the comments of Otaño et al. Our report was written in the context of two previous communications that depicted dire results from 5-FU exposure during pregnancy.[1,2] We could not find any report of a healthy pregnancy outcome. The patients referred to our center had been so advised by their physicians. Both patients considered early pregnancy termination in light of such a poor prognosis.

We shared the little and very concerning literature with our patients and, as is outlined in our article, monitored them closely. One patient accepted amniocentesis, and both patients underwent ultrasonographic targeted imaging to identify any suggestion of fetal malformation. Both infants were healthy. In this context, we felt it important to report these two cases to add balance to the published reports.

Periconceptual exposure to 5-FU in the United States appears to be a relatively rare event. In our two genetic centers we have not seen any other case to date. We know of five other cases, all of which have resulted in healthy pregnancies (Butler WJ. Personal communication). The letter from Otaño et al. adds important information to this field and again emphasizes the fact that healthy infants may result from these instances of periconceptual 5-FU exposure. We hope Otaño et al. publish their data in a full paper in the near future. We also hope that all cases of 5-FU exposure during pregnancy will be reported and recorded so the data can be compiled and analyzed.

It was clearly not our intention to suggest that periconceptual 5-FU exposure was an insignificant event. In view of the adverse outcomes reported to that point, we simply wanted to report two healthy births. The most important step in this issue is to prevent such events from occurring in the first place by ensuring that patients not get pregnant during exposure cycles. In those cases in which exposure occurs we advocate close monitoring via early fetal imaging with high-resolution vaginal ultrasonography and discussion with the patients as to the indications for fetal chromosomal

studies. We feel this approach is desirable at this time given the lack of solid data on this complex issue.

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Criteria for the diagnosis of infertility

To the Editors: We read with great interest the article by Aitken et al. (Aitken RJ, Irvine DS, Wu FC. Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. AM J OBSTET GYNECOL 1991;164:542-51). We believe it presents an exciting data set that provides clear evidence of the validity of the assessment of sperm-oocyte fusion and reactive oxygen species generation in the assessment of male fertility when conventional semen analysis is of no prognostic value.

Recently published data by Aitken and West[1] and by us[2] have put forward the concept of the contribution of seminal leukocytes to the levels of reactive oxygen species detected in semen. In view of those data and because the procedure used to prepare the semen samples (centrifugation three times) in the study of Aitken et al. yields varying numbers of these seminal elements, which are present in almost every semen sample,[3] we believe that an important question should be addressed. Have the authors screened the examined semen samples for leukocyte numbers and types? If available, these data will shed new light on the role of seminal leukocytes on the function of spermatozoa and subsequent fertility. Furthermore, such information will help decide the therapeutic approach to treat men with reactive oxygen species–related infertility. Because evidence from in vitro experiments supports the inhibition of reactive oxygen species–induced lipid peroxidation,[4] in vivo treatment with this antioxidant in cases of spermatozoa-derived reactive oxygen species might be beneficial. However, if leukocytes are the main source of reactive oxygen species, treatment with antibiotics might be more appropriate.

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Reply

To the Editors: I am grateful to Kessopoulou et al. for their interest in our studies on the diagnostic significance of the hamster oocyte penetration test and measurements of reactive oxygen species in cases of male infertility. I fully concur that the therapeutic implications of the reactive oxygen species data will be influenced by a knowledge of the source of the reactive oxygen species, although there are limitations to the value of this information.

Within the data set described in our article we found no significant relationships between the presence of round cells in the washed ejaculate and the level of reactive oxygen species production. However, we did not definitively identify or type the leukocyte populations in these samples with the immunocytochemical techniques described in our recent publication.[1] Where such techniques have been applied,[2] we showed that all human semen samples contained leukocytes and that the low levels of reactive oxygen species production observed in the normal fertile population were highly correlated with the concentration of these cells. Within our semen donor panel we have even found a case in which normal fertility was apparently compatible with high levels of reactive oxygen species production associated with concentrations of seminal leukocytes that were tenfold higher than the 1×10^6/ml threshold of normality suggested by the World Health Organization (1987). Such a situation could arise if activated leukocytes only enter the seminal fluid at the moment of ejaculation (from the seminal vesicles or prostate gland, for example), in which case the reactive oxygen species generated by the leukocyte population would not damage the spermatozoa because the spermatozoa would be protected by the antioxidant properties of seminal plasma.[3] Conversely, if activated leukocytes enter the male reproductive tract at a more proximal site, such as the rete testes or epididymis, the opportunity for inducing peroxidative damage in spermatozoa would certainly exist. In other words, the clinical interpretation of leukocytospermia is not straightforward; it is confounded by a number of factors, including the site of infiltration, the leukocyte species involved, and the nature and kinetics of their activation, none of which can be ascertained by simple leukocyte counts.

Furthermore, generation of reactive oxygen species by leukocytes should not be confused with generation of reactive oxygen species by spermatozoa, which is highly correlated with the appearance of defective sperm function.[4, 5] Therefore I reinforce the recommendation by Kessopoulou et al. that in cases of elevated reactive oxygen species production the source of

the oxidative stress be identified to differentiate cases involving leukocytic infiltration from those associated with a defect at the level of the germ cell. A protocol for achieving this distinction is in press.[2]

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Anticancer drugs during pregnancy: Are we able to discard them?

To the Editors: We read with interest the article by van der Zee et al. (van der Zee AGJ, de Bruijn HWA, Bouma J, Aalders JG, Oosterhuis JW, de Vries EGE. Endodermal sinus tumor of the ovary during pregnancy: A case report. Am J Obstet Gynecol 1991;164:504-6). They treated a patient with endodermal sinus tumor surgically and without chemotherapy during pregnancy. She received a cisplatin-containing regimen after delivery. van der Zee et al. stated the potential risks of cisplatin during pregnancy and the well-known efficacy of cisplatin-containing regimens. However, chemotherapy other than cisplatin can be used in this well-known malignant germ cell tumor during pregnancy.

The potential risks of cisplatin are already well described, which was why we used vincristine, actinomycin D, and cyclophosphamide (VAC) instead of cisplatin-containing regimens during pregnancy in a recent case report.[1] In the case we reported, the patient had received conservative surgery and six courses of VAC combination chemotherapy from 16 weeks' gestation and was delivered of a normal infant at 37 weeks' gestation. After delivery we replaced the VAC protocol with a vinblastine, bleomycin, and cisplatin protocol. Two courses of this cisplatin-containing regimen were used. The patient and her child are doing well >5 years after treatment.

Endodermal sinus tumor of the ovary appears to be almost uniformly fatal; most authors report patient survival with a 2- or 3-year survival rate rather than a 5-year survival rate. Although van der Zee et al. believe cisplatin-containing regimens improve the prognosis of endodermal sinus tumor, the usefulness of VAC is not fully discarded. The promising results achieved with VAC were confirmed by others, at least in patients with early stage disease.[2]

van der Zee et al. avoided giving chemotherapy during pregnancy; however, there are numerous reports concerning micrometastasis in early stages of ovarian cancers. Although we do not know whether the endodermal sinus tumor propagates or remits during pregnancy, who is responsible for advancement of the disease if there are lymph node metastases or if invisible masses remain after surgery? Can we ignore the possibility of metastasis during pregnancy?

There is a therapeutic dilemma for the pregnant patient in whom is found a malignancy that requires chemotherapy. As yet chemotherapy in the second and third trimesters has not resulted in teratogenicity.[1] We believe it to be necessary to use non-cisplatin-containing regimens of chemotherapy during pregnancy, at least in this highly malignant tumor of the ovary.

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Reply

To the Editors: We thank Kim et al. for their interest in our case report. We agree that an unexpectedly low incidence of teratogenesis is associated with the use of anticancer agents in pregnancy, especially when used in the second and third trimesters. However, the experience is limited: The literature consists mainly of case reports (mostly successful); hardly any information is available to determine the long-term risks of in utero exposure to chemotherapy.[1,2] In an overview of adult cancer survivors, Loescher et al.[3] described a higher risk of second malignancies in patients after receiving chemotherapeutic agents. This higher risk is especially the case after alkylating agents, such as cyclophosphamide, which was used by Kim et al. So far no comparable data are available to determine long-term effects for individuals who have been exposed to chemotherapy in utero. Kim et al. are concerned about metastases of endodermal sinus tumor to the fetus. However, to date only seven documented cases of fetal metastases of other malignancies have been described.[1] Considering these data, the risk for fetal metastases seems to be very low and is hardly an argument for starting chemotherapy during pregnancy. The maternal serum α-fetoprotein is a sensitive and well-established marker in endodermal sinus tumor. Weekly measurement of the maternal serum α-fetoprotein enabled us to detect

the recurrence of endodermal sinus tumor at an early stage. We awaited further maturation of the child until caesarean section was done in the 33rd week of gestation. Kim et al. chose to treat their patient during pregnancy with a regimen of vincristine, actinomycin D, and cyclophosphamide (VAC) to avoid the use of cisplatin during pregnancy. In a recent review Nash and Young[4] stated that, although only randomized prospective trials can definitively establish survival advantages between the VAC and PVB (cisplatin, vincristine, and bleomycin) regimens, the cumulative experience from the existing literature seems to indicate that PVB is a more active regimen.[4,5] These authors tend to prefer the BEP (cisplatin, etoposide, and bleomycin) regimen, which we used in our patient, because the results are comparable to those of PVB but with less long-term toxicity.[4] We were fully aware of a high risk of recurrence of the endodermal sinus tumor after surgical resection without adjuvant chemotherapy. However, the availability of cisplatin-containing regimens with well-known high efficacy, which result in survival rates of 96% in stages I and II and 79% in stages III and IV, enabled us to postpone chemotherapy until after the delivery of the child.[4] We were able to treat the mother with the chemotherapeutic regimen with the highest efficacy and to omit unknown potential carcinogenic damage to the fetus. To date, the mother and her 4-year-old child are doing well.

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Benefits of cesarean section stapling device understated?

To the Editors: I read the article by Villeneuve et al. (Villeneuve MG, Khalifé S, Marcoux S, Blanchet P. Surgical staples in cesarean section: A randomized controlled trial. Am J Obstet Gynecol 1990;163:1641-6) with interest. However, a few points need further clarification. Although the instrument depicted in Fig. 1 is the device used in their study, it is no longer available in Canada and has never been available in the United States. Also, although Dr. Gilles Monif is a well-respected member of the obstetrics and gynecology community specializing in the treatment of infectious disease, his criteria for morbidity are not universally known, and a reference or description of his criteria for endometritis would be helpful.

The study is an excellent investigation in the use of the Auto Suture Poly CS* 57 disposable surgical stapler. Although minor questions arise, such as the length of experience of the instrument's users, the study confirms the findings of earlier reports.[1-4] However, the conclusions of Villeneuve et al. are not substantiated by or consistent with their own and previously reported data. Their conclusions are perhaps different because of their failure to recognize that their population may represent a specific subset of the cesarean section population in North America. Their results do not contradict those of Burkett et al. because the patient populations had several differences: Burkett et al. found a significant decrease in infectious morbidity presumably caused by their higher baseline postoperative infection rate. The decrease in postoperative infection with the use of the cesarean section stapler was significant and led to considerable cost savings. The population of Villeneuve et al. does not have a high infection rate; therefore it would take a larger number of patients to demonstrate significant benefit. However, the decrease in blood loss in the study of Villeneuve et al. was significant; I disagree with their conclusion that significant savings of blood is not beneficial. Similar studies have been performed in a low-risk infectious population, and similar significant decreases in blood loss were found.[1,3] This decrease in blood in the operative field was of major benefit to the operating physicians, turning a usually bloody procedure into a safer and easier procedure for the surgeon. The significant decreases in blood loss are also beneficial to the patient in light of the decreased but ever-present risk of transfusion-related human immunodeficiency virus and other infectious exposure. Other studies in high-risk infectious populations have demonstrated >60% decrease in postoperative infection rates with the surgical stapling device in high-risk populations similar to those studied by Burkett et al.

As mentioned by Villeneuve et al. the retaining pin had the potential for causing vessel puncture, and it has been removed in the new Food and Drug Administration–approved Auto Suture Poly CS 57 instrument. Therefore, although I thought the study of Villeneuve et al. was well done, I do not agree with their conclusion that significant decreases in blood loss are not important enough to give the instrument further consideration. Their patient population was at low risk for postoperative infections; therefore their sample size

*Reg. U.S. Patent and Trademark Office. Auto Suture Company, Division of United States Surgical Corporation, Norwalk, Conn.

was not large enough to allow them to make comments regarding the instrument's role in reducing post–cesarean section infections. Thus the question of the utility of the Auto Suture Poly CS 57 disposable uterine device still deserves further attention.

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The uterine stapling device for cesarean section: A positive view

To the Editors: In their study comparing the Auto Suture Poly CS 57 disposable uterine stapler with the standard hysterotomy for cesarean section, Villeneuve et al. did not find reductions in operating time, risk of febrile morbidity, or length of postoperative hospital stay in the group of patients in whom the uterine stapler was used. In addition they considered that the difference in blood loss between both groups was small and of questionable clinical significance and that their results do not support the routine use of the Auto Suture Poly CS 57 in low transverse cesarean sections.

I would like to comment on this article. The authors state that theirs is the first randomized study comparing the uterine stapling device with the standard suture technique for cesarean deliveries in human beings. They also mention that only one previous study, which was not a randomized controlled trial,[2] had evaluated the Auto Suture Poly CS 57. Their statement is inaccurate. Human studies evaluating the staple device have appeared since 1988; several are randomized controlled trials.[3] All investigators found the uterine stapler principle to be unquestionably sound, and no comparative series provided an overall statistically superior result in the traditional suturing technique when compared with the uterine stapler.

On the basis of the available clinical data it can be concluded that the benefits of the absorbable uterine stapler depend on surgical experience and patient population. Significant reductions in blood loss and operating time can be expected in the more experienced surgeons who are familiar with the device. In high-risk obstetric patients the instrument produces a significant decrease in the rate of febrile morbidity, resulting in a shorter hospital stay and a substantial savings in hospital costs. The socioeconomic characteristics and obstetric risk factors of the study conducted by Villeneuve et al. were not stated in their article.

It is difficult to understand the authors' high failure rate regarding the correct application of the uterine stapler (45.5%) and their high incidence of accidental extensions (42%) and associated significant bleeding (62%), which obviously stems from their incapability of completely applying the stapler. Failure to use the entire length of the stapler cartridge will result in an incision smaller than the required 11.5 cm. In light of this, their estimated total blood loss in the cases of the study is inaccurate because no differentiation in volume of blood loss can be made between that of the uterine edges where the staples were applied and of the traumatic uterine wall bleeding.

In my experience with >100 cases, a learning curve of 3 to 6 cases seems necessary to maximally use the stapling device.[4]

I concur with the author's conclusion that it is probably premature to recommend routine use of the uterine stapler for every cesarean section. Nevertheless, on the basis of current information, its use should be strongly recommended for obstetric patients in whom the expectation of blood loss during the cesarean section is greater than average, the amount of intraoperative blood loss is critical, or the risk of postoperative infectious morbidity is high.

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To the Editors: Thank you for this opportunity to clarify some aspects of our randomized clinical trial on the Auto Suture Poly CS 57 staples used in cesarean sections.

We considered febrile morbidity to be present when at least one of the following conditions was satisfied[1]: (1) fever ≥38° C on two consecutive occasions ≥4 hours apart, with the second reading ≥0.2° C higher than the first; (2) fever ≥38.5° C on one occasion; or (3) fever ≥38° C on three occasions in any 24-hour period.

We do not think our conclusions would have been different had our experience with the instrument been longer. Data were reanalyzed for the first (period 1) and last (period 2) 100 patients enrolled in the study separately (Table I). Results are largely similar for the two periods. In period 2, when obstetricians had gained

Table I. Blood loss and operating time according to hysterectomy procedure, stratified for study period

	Period 1		*Period 2*	
	Stapling device	*Standard hysterotomy*	*Stapling device*	*Standard hysterotomy*
Blood loss				
Total blood loss (ml, mean ± SEM)	517 ± 33	568 ± 59	466 ± 35	588 ± 48
Blood loss caused by hysterotomy (ml, mean ± SEM)	275 ± 29	337 ± 51	203 ± 26	253 ± 32
Hemoglobin deficit (gm/dl, mean ± SEM)	1.06 ± 0.1	1.23 ± 0.1	1.25 ± 0.2	1.24 ± 0.2
Operating time (min, mean ± SEM)				
From uterine incision to closure of hysterotomy	13.1 ± 0.6	11.3 ± 0.7	12.4 ± 0.6	13.0 ± 0.7
From abdominal incision to closure of the wound	41.3 ± 1.6	38.0 ± 1.7	43.7 ± 1.9	40.2 ± 1.6

experience with the stapler, the reduction in total blood loss with the stapler was still minimal (122 ml), and no difference emerged in the hemoglobin deficit. Moreover, the use of the stapler did not substantially reduce the operating time.

We agree that significant savings of blood are beneficial for the patients. However, the magnitude of the difference we observed in total blood loss was small, and the hemoglobin deficit was similar in the two groups. Therefore we feel that the small differences observed in estimated blood loss had limited clinical significance.

We did not observe a beneficial effect of the instrument on the incidence of endometritis. Given the low baseline incidence of this complication in our study population, we agree that such an effect would be statistically difficult to detect. In our view the use of the stapling device with the main objective of reducing the number of women with endometritis does not seem warranted in a population in which the baseline frequency of endometritis is as low as in ours.

We are pleased that the retaining pin in the stapler we tested was subsequently removed. This pin complicated the surgery in six of our patients. The removal of the pin is certainly an improvement over the original instrument.

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The short umbilical cord

To the Editors: A short umbilical cord can predispose a fetus to distress, rupture of the umbilical cord, premature placental separation, failure of the presenting part to descend, and primarily uterine inversion. A short cord with a vas insertion is a rare and deadly combination. In 1750 Smellie[1] described a fetal death caused by a short cord. DeLee[2] summarized short cord literature and established a length of 25 cm and fundal cord insertion as an absolute definition. Gardiner[3] attempted to redefine a short umbilical cord as ≤32 cm in length. Rosen[4] reviewed the literature of his day and found the incidence of a short umbilical cord to be between 0.78% and 0.43%. He distinguished between the absolutely short umbilical cord (an average of <30 cm) and the relatively short umbilical cord (attributed to cord entanglement). The potential danger of such umbilical cord presentations remains in question. Rosen concludes that stillbirth is not increased in the absolutely short cord group versus the control group. Recently Sørnes attempted to evaluate short cord effects on delivery and believed it to be "hazardous for the infant."[5] He concludes that "two thirds of the babies with cords less than 35 cm length were born nonoperatively and this is unexplained."

The explanation is provided by Brickner: "the placental location and placental umbilical cord insertion must be considered"[6] when studying short cords. When a placenta is low lying and has a battledore insertion of the umbilical cord near the cervix, a 20 cm umbilical cord is ample for delivery of the infant to the umbilicus. A short cord can be suspected if the placenta is known to be fundally located and the umbilical cord is inserted centrally, creating the longest length necessary to deliver. With a floating presenting part and failure of descent during labor combined with possible signs of fetal distress, a potential short cord complication can be avoided.

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Crown-rump length dating of pregnancy at less than nine weeks' gestation

To the Editors: I have used the following formula for the last 7 years to date intrauterine pregnancy at <9 weeks' gestation by using crown-rump length when embryonic cardiac activity is present: Mean gestational age (menstrual age) in days = 40 + The crown-rump length in millimeters (e.g., 9 mm crown-rump length = 49 days or a 7-week embryo). This formula suggests that there is linear growth of the embryo (about 1 mm per day) until 9 weeks of gestation or until the embryo is approximately 25 mm or 1 inch in length. The 95% confidence limits are ±4 days assuming the pregnancy is normal and the crown-rump length measurement is accurate.

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First successful cesarean section in the British empire

To the Editors: Gabert and Bey[1] recently reviewed the history and development of cesarean section in an interesting and informative manner. One such operation that has escaped general attention, however, involves a legendary figure of nineteenth-century medicine.

James Miranda Stuart Barry (1795-1865) entered Edinburgh University in 1809 and qualified as a doctor of medicine in 1812.[2-4] Because Barry completed the requirements at an early age, the senate of the university refused to admit him to the degree until political pressure was brought to bear.

On July 5, 1813, Barry entered the British army as a medical assistant over the objection of the principal medical officer. In 1815, after he had been posted to the Cape of Good Hope, his skill as a physician was soon recognized and he was frequently sought as an obstetrician.

Sometime between 1815 and 1821, Barry performed a cesarean section on a patient named Mrs. Munnik. It is not known why the cesarean was required. Both mother and child, James Barry Munnik, survived the operation. A grandson of this child, James Barry Munnik Hertzog (1866-1942), later became the prime minister of South Africa from 1924 through 1939.

Barry learned about obstetrics from a course at Edinburgh given by James Hamilton (1767-1839), professor of midwifery at the university and the acknowledged leader in obstetrics in Scotland. Although midwifery was not required for graduation, Hamilton's course and teaching sessions at the hospital were always crowded.[5] Hamilton performed two cesarean sections but had fatal results in both instances.

When Barry died in London in 1865 it was discovered that she was a woman. From 1809 to 1865 she had successfully masqueraded as a man. She is now recognized as the first British woman physician and the first physician of either sex in the British empire to perform a cesarean section in which both the mother and the child survived.

The first successful operation in America was performed by Jesse Bennett on his wife in 1794.[6] King[7] maintains that the honor belongs to Richmond, who operated in 1827. Gabert and Bey[1] support Richmond's claim; therefore some question still exists about who was first. Incidentally, Richmond was forced to sacrifice the child to save the mother. Barry's section took place between Bennett's and Richmond's.

Two other reports of operations in this period deserve passing comments. Naqui,[8] contending that Barlow performed a successful operation in 1793, is in error because although the mother was saved the child was dead. In 1834 Greaves[9] saved both mother and child. The operation of Barry clearly preceded that of Greaves.

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Roster of American obstetric and gynecologic societies*

(appears annually in the January issue)

American Board of Obstetrics and Gynecology, Inc. (1930) President, Albert B. Gerbie. Secretary, James A. Merrill, Executive Director, 4225 Roosevelt Way, N.E., Suite 305, Seattle, WA 98105.

American Gynecological and Obstetrical Society. President, Leo Dunn. Secretary, James R. Scott, Department of Obstetrics and Gynecology, 50 N. Medical Dr., Salt Lake City, UT 84132. Meeting, Sept. 10-12, 1992.

Central Association of Obstetricians and Gynecologists. (1929) President, John J. Sciarra. Secretary, Karl C. Podratz, Mayo Clinic, W10B, 200 First St., SW, Rochester, MN 55905. Meeting, Oct. 15-17, 1992.

Pacific Coast Obstetrical and Gynecological Society. (1931) President, Walter S. Kiefer. Secretary, Theodore W. Loring, 1751 East Wood Dr., Eureka, CA 95501. Meeting, Oct. 11-16, 1992.

Society for Gynecologic Investigation. President, Frederick Naftolin. Secretary, Rogerio A. Lobo, Department of Obstetrics and Gynecology, Women's Hospital, Room 1M2, LAC/USC Medical Center, 1240 N. Mission Road, Los Angeles, CA 90033. Meeting, March 18-21, 1992.

Society of Perinatal Obstetricians. President, Garland Anderson. Secretary, Sze-ya Yeh, Department of Obstetrics and Gynecology, Allentown Hospital, 17th and Chew Streets, Allentown, PA 18102. Meeting, Feb. 3-8, 1992.

South Atlantic Association of Obstetricians and Gynecologists. (1938) President, Willette L. LeHew. Secretary, W. Glenn Hurt, Medical College of Virginia, Box 34, Richmond, VA 23298. Meeting, Jan. 26-29, 1992.

American Association of Pro Life Obstetricians and Gynecologists. President, David V. Foley, MD. Secretary, Robert H. Herzog, Executive Director, 850 Elm Grove Rd., Elm Grove, WI 53122. Meeting, Monday, April 27, 1992 (during ACOG annual meeting).

American College of Obstetricians and Gynecologists. (1951) President, Richard H. Schwarz. Secretary, Mary Jo O'Sullivan, 409 12th St., S.W., Washington, DC 20024. Meeting, April 26-30, 1992.

*Changes, omissions, and corrections must be received by the publisher two months in advance (by November 1) for the Roster. Please address the Journal Editing Department, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318. The number after the society's name is the year of founding. For further information, address the respective secretaries.

American Society for Psychosomatic Obstetrics and Gynecology. (1971) President, Regina P. Lederman. Secretary, Cheryl F. McCartney, 409 12th St., SW, Washington, DC 20024-2188, Attn: Kathleen Graham. Meeting, Feb. 26–March 1, 1992.

Brooklyn Gynecological Society, Inc. President, Frederick H. Sillman. Secretary, Jack E. Maidman, Long Island College Hospital, Brooklyn, NY 11201. Meetings, Jan. 15, Feb. 19, April 15, and May 20, 1992.

Chicago Gynecological Society. (1878) President, Ulisse P. Cucco. Secretary, Pedro A. Poma, 675 W. North Ave., Suite 407, Melrose Park, IL 60160. Meetings, Jan. 17, Feb. 21, March 20, April 17, May 15, and June 19, 1992.

Cleveland Society of Obstetricians and Gynecologists. President, James J. Izanec. Secretary, Robert Kiwi, c/o Alexander W. Kennedy, 9500 Euclid Ave., Cleveland, OH 44195. Meetings, Jan. 15, March 18, and May 20, 1992.

Columbus Obstetric and Gynecologic Society. (1945) President, Jeffrey Bell. Secretary, Charles A. Caranna, 1215 Brittany Ln., Columbus, OH 43220. Meeting, fourth Wednesday of each month, September-June.

Florida Obstetric and Gynecologic Society. President, Paul A. Gluck. Secretary, Thomas K. Young, 6440 W. Newberry Rd. #508, Gainesville, FL 32605-4380.

Milwaukee Gynecological Society. (1951) President, Charles Dungar. Secretary, Robert H. Herzog, Executive Director, 850 Elm Grove Rd., Elm Grove, WI 53122. Meetings, first Tuesday of October and November; second Monday of February, March, and April.

Nassau Obstetrical and Gynecological Society, Inc. President, Stuart Leon Bednoff. Secretary, Louis J. Iandol, 2000 Lakeville Rd., New Hyde Park, NY 11040. Meetings, Feb. 10 and April 13, 1992.

North American Society for Pediatric and Adolescent Gynecology. (1986) President, Gita P. Gidwani. Secretary, Susan Porkorny, 1015 Chestnut St., Suite 926, Philadelphia, PA 19107. Meeting, April 2-4, 1992.

Northeastern New York Obstetrical and Gynecological Society. President, Katy Irani. Secretary, Robert H. Dropkin, Executive Park East, Stuyvesant Plaza, Albany, NY 12203. Meetings, Jan. 16, April 30, and Sept. 24, 1992.

Pittsburgh Obstetrical and Gynecological Society. President, James H. Harger. Secretary, Robert S. Blockstein, 4815 Liberty Ave., Pittsburgh, PA 15224. Meetings, Feb. 3, March 2, April 6, and May 4, 1992.

San Francisco Gynecological Society. (1929) President, James Stickler. Secretary, Gerald Shefren, 1101 Welch Rd., Palo Alto, CA 94304. Meetings, Jan. 14, Feb. 11, March 10, April 14, and May 12, 1992.

Wisconsin Society of Obstetrics and Gynecology. President, Paul Harkins. Secretary, Robert Herzog, Executive Director, 850 Elm Grove Rd., Elm Grove, WI 53122. Meeting, July 16-18, 1992.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Common Problems in Obstetric Care II, April 20-25, 1992, Westin Maui, Maui, Hawaii. Sponsored by the Departments of Obstetrics and Gynecology, Cornell University Medical College–The New York Hospital and The University of Hawaii Medical Center. For information contact: Colette Carmeris, Course Coordinator, Department of Obstetrics and Gynecology, NYH-CUMC, 525 East 68th St., New York, NY 10021. Tel.: (212) 746-3059.

Issues in Obstetrics and Gynecology, February 3-7, 1992, The Hyatt Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, PA/C, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

4th Annual Review Course in Obstetrics & Gynaecology, May 6-9, 1992, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA Category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

Office Gynecology, April 20-24, 1992, The Hyatt Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, PA/C, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

The Annual International Reproductive Health Seminar, October 29-31, 1992, Cocoa Beach, Florida. Sponsored by IPARC (International Population and Reproduction Council), Inc., in cooperation with IPARC Human Development Program, Inc. For further information contact: Howard A. Engle, MD, Director General, IPARC, 975 Arthur Godfrey Road, Suite 102, Miami Beach, FL 33140. Tel.: (305) 531-0047. Outside IPARC North American Region (U.S. and Canada) contact: Arnaldo De Moraes II, MD, PhD, Vice President, IPARC, 20 Princesa Januaria St., Flamengo 22250, Rio de Janeiro, R.J., Brazil. Tel.: (021) 552-7349.

Advanced Diagnostic & Therapeutic Techniques in Obstetrics and Gynecology: A Hands-on Seminar, March 11-14, 1992, Cliff Lodge, Snowbird, Utah. For information, contact The Graduate School of Medicine, Registration Office, 707 South Wood St., Chicago, IL 60612. Tel.: (800) 621-4651.

June 8, 1992: 2nd International Symposium on Perinatal Asphyxia: Current Concepts and Intrapartum Fetal Surveillance. Presented by The Canadian Medical Protective Association in conjunction with The Society of Obstetricians and Gynaecologists of Canada, at the Westin Bayshore, Vancouver, British Columbia. Contact: Mrs. Joan Beards, C.M.P.A. Education Department, Perinatal Asphyxia Conference, P.O. Box 8225, Ottawa, Ontario, Canada K1G 3H7. Tel.: (613) 236-2100 (ext. 238); fax: (613) 236-5588.

The 3rd Annual Conference on The Prevention of Prematurity and Neonatal Mortality, March 26-28, 1992, at the Westin Peachtree Plaza in Atlanta, Georgia. Sponsored by Emory University School of Medicine, with an educational grant from Healthdyne, Inc., Department of Professional Education. Conference Chair: John Huddleston, MD. For further information contact Dr. McEntire at (800) 366-2229 or Emory Office of Continuing Medical Education at (404) 727-5695.

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The Department of Obstetrics and Gynecology at the University of Pittsburgh is seeking a full-time faculty member in the Division of Reproductive Genetics.

Applicants must be Board-Certified in Obstetrics and Gynecology and Board eligible or certified in Human Genetics. Additional subspecialty eligibility/certification in Maternal-Fetal Medicine is preferred. Applicants should demonstrate interest and experience in basic and/or clinical research, undergraduate and graduate education, and clinical practice.

Academic rank and salary will be commensurate with experience, training and achievements.

Send inquiry and curriculum vitae to:

Richard L. Sweet, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
University of Pittsburgh School of Medicine
c/o Magee-Women's Hospital
300 Halket Street
Pittsburgh, PA 15213

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FACULTY POSITION
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The Department of Obstetrics & Gynecology at the University of Kansas School of Medicine-Wichita is accepting applications for the position of Director of the Division of Maternal-Fetal Medicine. This represents a unique opportunity to have the best features of both academic medicine and private practice.

Please send curriculum vitae to:

Daniel K. Roberts, M.D., Ph.D.
Professor & Chairman, Dept. of Ob/Gyn
University of Kansas School of Medicine-Wichita
HCA-Wesley Medical Center
550 N. Hillside
Wichita, KS 67214

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The University of Manitoba

POSTGRADUATE FELLOWSHIP MATERNAL & FETAL MEDICINE

Applications are invited for a two-year Fellowship in Maternal & Fetal Medicine under the direction of the Section of Maternal & Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, Dr. F.A. Manning, Professor. This fellowship, which is accredited by The Royal College of Physicians and Surgeons of Canada, is available July 1, 1992.

The successful applicant will receive a comprehensive training experience in all aspects of high-risk obstetrics. Specific instruction in the real-time ultrasound assessment of the fetus using the biophysical profile score, intrauterine transfusion, fetal surgery, and experimental fetal research are an integral part of the program.

The University of Manitoba encourages applications from qualified women and men, including members of visible minorities, aboriginal people, and persons with disabilities. The University provides a smoke-free work environment.

Applicants to this program should possess Royal College of Physicians and Surgeons certification in Obstetrics and Gynecology or its equivalent. Applications including full C.V. and three professional references should be sent to: C.R. HARMAN, M.D., DIRECTOR, FELLOWSHIP PROGRAM, SECTION OF MATERNAL AND FETAL MEDICINE, UNIVERSITY OF MANITOBA, 735 NOTRE DAME AVENUE, ROOM WR120, WINNIPEG, MANITOBA, R3E, OL8, CANADA.

GYNECOLOGIC ONCOLOGIST

The Department of Obstetrics and Gynecology at the University of Massachusetts Medical Center is seeking a third gynecologic oncologist. Applicants should be Board-eligible or certified in Obstetrics and Gynecology and Gynecologic Oncology, and also must be eligible for licensure in the State of Massachusetts. The Department is active in clinical research. Many opportunities exist for collaboration in basic research areas. Candidates should have an interest in medical student and resident education. Academic rank and salary will be based on experience. UMMC is an AA/EOE employer.

Interested candidates should send a curriculum vitae and letter to:

Kenneth L. Noller, M.D. and Chairman
UMMC
55 Lake Avenue North
Worcester, Ma 01655

Director of Reproductive Genetics

We seek an ABMG-certified or -eligible reproductive geneticist to lead the Department of Reproductive Genetics of the Magee-Womens Hospital, with a faculty appointment at the appropriate level in the University of Pittsburgh's Department of Human Genetics and membership in the Pittsburgh Genetics Institute. Research potential is important. Send CV and letter of interest and research goals to John J. Mulvihill, M.D., Department of Human Genetics, University of Pittsburgh, 130 DeSoto Street, Pittsburgh, PA 15261. The University of Pittsburgh and Magee-Womens Hospital are Equal Opportunity/Affirmative Action Employers.

UMD
NEW JERSEY

DIRECTOR
MATERNAL FETAL MEDICINE
Department of Obstetrics & Gynecology
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School

The Robert Wood Johnson Medical School invites applications for **Director of Maternal-Fetal Medicine.** The position of Director requires an individual with an established and broad understanding of medical education, research, patient care as well as effective administrative skills. Applicants should possess qualifications commensurate with those of a Professor of Obstetrics and Gynecology as defined by the Robert Wood Johnson Medical School.

The Division is located at St. Peter's Medical Center, the largest obstetrical and neonatal program in the State. Maternal-Fetal Medicine consists of five perinatologists, four sonographers, geneticist, two research nurses and one epidemiologist/biostatistician. The Robert Wood Johnson Program supports 7,000 deliveries per year.

Interested individuals should forward their curriculum vitae to: **Robert A. Knuppel, M.D., M.P.H., Professor and Chairman, Department of Obstetrics & Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, CN 19, New Brunswick, NJ 08903-0019.**

The UMDNJ is an Affirmative Action/Equal Opportunity Employer M/F/H/V and a member of the University Health System of New Jersey.

UMD
NEW JERSEY

Department of Obstetrics and Gynecology

University of California Irvine, College of Medicine, Obstetrics and Gynecology academic position: Associate Professor In-Residence/Professor In-Residence (M.D.), Director, Division of Gynecology, board certified. Extensive clinical experience in the management of ambulatory gynecology and gynecologic surgery. Previous administrative experience and special interest in infectious diseases or contraception desirable. Duties include medical student, resident, and postgraduate teaching, clinical service supervision, clinical and laboratory research. Private practice optional. UCI is an Equal Opportunity/Affirmative Action Employer. Send C.V. and five references to Thomas J. Garite, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, UCI Medical Center, 101 The City Drive, Orange, CA 92668.

State University of New York
Health Science Center
Syracuse

MATERNAL-FETAL MEDICINE SUBSPECIALIST

The Department of Obstetrics and Gynecology of the SUNY Health Science Center at Syracuse is actively recruiting a fifth full-time Perinatologist for its Division of Maternal-Fetal Medicine.

The teaching hospital serves as the only Tertiary Care Center for the Regional Perinatal Program encompassing the 15 counties of Central New York with a population of 2.3 million.

Excellent opportunity to participate in teaching in a University based setting and collaborate in ongoing research in clinical Perinatology, Ultrasound, and Prenatal Diagnosis.

TOP SALARY, ATTRACTIVE BENEFITS PACKAGE, AND PROTECTED RESEARCH TIME.

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Interested candidates should send curriculum vitae to:

Robert K. Silverman, M.D.
Division of Maternal-Fetal Medicine
SUNY Health Science Center at Syracuse
Perinatal Center
725 Irving Avenue, Suite 115
Syracuse, NY 13210
(315) 470-7193

SUNY Health Science Center is an equal opportunity, affirmative action employer.

PERINATOLOGIST

The Stamford Hospital, a 300+ bed voluntary hospital located in Northeast currently seeking a board-certified or board-eligible **Perinatologist** to work in a fully-equipped, well-developed unit in a fully-accredited residency program. Faculty appointment to affiliated medical school available. Competitive compensation and benefits program. Please submit CV to: Dr. Morton A. Schiffer, Obstetrician and Gynecologist in Chief, The Stamford Hospital, P.O. Box 9317, Shelburne Rd. at West Broad St., Stamford, CT 06904-9317.

The Stamford Hospital

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THE HEALTH CENTER FOR WOMEN

St. Paul-Ramsey Medical Center
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The Health Center for Women at St. Paul-Ramsey Medical Center and Ramsey Clinic is seeking out a physician specializing in obstetrics and gynecology to join the multi-disciplinary team at the Center. The Health Center for Women is a new approach to health care and was designed exclusively for women. Candidates should be board certified/eligible and possess excellent interpersonal skills. The Twin Cities of Minneapolis and St. Paul is considered one of the country's cleanest, safest and most enjoyable metropolitan areas! For more information, kindly forward your curriculum vitae to: **Loriese A. Stoll, Director of Professional Services, Ramsey Clinic, 640 Jackson Street, St. Paul, Minnesota 55101-2595, (612) 221-3067.**

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OB/GYN BC/BE position available in Oklahoma City. New fully equipped, LDRP facility with central fetal monitoring, telemetry, dedicated staff, 24 hour obstetrical anesthesia and family prenatal classes. Level II nursery. Strong pediatrician support. Malpractice rates reasonable. Call coverage. Salary and benefits guaranteed. Reply with CV to Medical Services Rm. 3129, P.O. Box 205, Oklahoma City, OK 73101.

OB/GYN

St. Elizabeth Medical Center is offering an excellent opportunity to a bright and talented OB/GYN physician. Our JCAHO facility in Northern Kentucky is ideally located close to the Cincinnati area. This opportunity will offer a teaching environment, possibilities for clinical research and hands on practice. We are a Level II facility handling an average of 3000 deliveries per year. If you are tired of private practice and working from 8-5 sounds good (on call one night per week), this is the position for you.

For more information please contact our Physician Services Director:

Joni Yeager
St. Elizabeth Medical Center
401 E. 20th Street
Covington, Kentucky 41014
(606) 292-4023

PERINATOLOGIST

St. Joseph's Hospital & Medical Center, affiliated with the University of Medicine & Dentistry of New Jersey and Seton Hall University School of Graduate Medical Education, is seeking a fourth BC/BE specialist in Maternal-Fetal Medicine for its Regional Perinatal Center. Responsibilities include patient care, teaching, active perinatal referral center. Private practice and research opportunities available. Please send resume to:

Pamela J. Tropper, MD, Director, Maternal-Fetal Medicine, (201) 977-2499.

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UMD
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FELLOWSHIP IN PELVIC SURGERY
UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL

Department of Obstetrics and Gynecology
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School

A comprehensive one-year training program in pelvic surgery is being offered by the Division of Pelvic Surgery in the Department of Obstetrics and Gynecology at UMDNJ-Robert Wood Johnson Medical School.

The program is designed to train fellows in all aspects of gynecological surgery including: Perioperative Care, Radical Surgery for Gynecologic Malignancies, Urodynamic Evaluation, Treatment of Incontinent Women, Vaginal Reconstructive Endoscopic Pelvic Surgery, Surgical Intensive Care Management, Management of Breast Disease.

The fellow will be actively involved in a number of clinical research projects and will receive instruction in the design of clinical investigations and data analysis. The position is ideally suited for individuals seeking a career in academic gynecology.

Interested candidates should send their curriculum vitae to: **Nicholas Kadar, M.D., Director, Division of Pelvic Surgery, Department of Obstetrics & Gynecology, UMDNJ-Robert Wood Johnson Medical School, One Robert Wood Johnson Place, CN 19, New Brunswick, NJ 08903-0019.**

The UMDNJ is an Affirmative Action/Equal Opportunity Employer M/F/H/V and a member of the University Health System of New Jersey.

UMD
NEW JERSEY

Director of Perinatology

Due to expansion of the scope of the oldest and most prestigious family practice residency program in the south, we are seeking a BE/BC Perinatologist for the faculty/staff. With 36 residents, this progressive program's philosophy is to provide solid training in obstetrics. The position will be multi-faceted with aspects of patient care, teaching, and private consultation, and carries the opportunity for an academic affiliation with the Medical College of Georgia. There is a dedicated antenatal testing unit. The hospital is a 417-bed tertiary care facility with Level III NICU and 2 BC Neonatologists. Approximately 4000 births are facilitated each year, with 10% deemed high risk. Enjoy the luxury of excelling in your area of expertise with good call coverage from a large group of obstetricians who handle high risk patients as part of their daily practice. Highly competitive compensation package is offered. Just 90 minutes from Atlanta, this is the second largest city in Georgia, with a population of 250,000 and a 38 county referral base. To explore this opportunity, please contact:

Joyce Campbell
Physician Services of America
2000 Warrington Way, Suite 250, Louisville, KY 40222
(800) 626-1857, ext. 238

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF CALIFORNIA, DAVIS

A full-time position is available in the Department of Obstetrics and Gynecology, University of California, Davis, Medical Center, Sacramento. Applicants should be Board eligible or certified in Obstetrics and Gynecology. Applicants must possess or be eligible for medical licensure in the State of California. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities or potential for such activities.

Responsibilities of this position include inpatient and outpatient case management, participation in teaching activities with residents, interns and medical students, development and/or participation in research activities and involvement in University and Hospital committees.

Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
Sacramento, California 95816

The final date for receipt of applications is March 6, 1992.

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Medical College of Ohio
Faculty Positions

Director of Maternal Fetal Medicine
Director of Gynecologic Oncology
Director of Reproductive Endocrinology
Generalist in Obstetrics-Gynecology

The Department of Obstetrics-Gynecology at the Medical College of Ohio is actively recruiting outstanding professionals to fill important clinical positions as we are embarking on the rebuilding of the academic and patient service program.

The Medical College of Ohio has a modern, new ambulatory services facility, is involved in the community's only Level 3 perinatal center, and boasts a spectacular academic campus.

Qualifications include board eligibility or certification and a commitment to teaching. Salary and academic rank are commensurate in candidate's training and experience.

Send inquiries and curricula vitae to Louis Weinstein, M.D., Professor and Chairman, Department of Obstetrics-Gynecology, Medical College of Ohio, P.O. Box 10008, Toledo, Ohio, 43699.

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Send CV to: Oded Rencus, MD., Chief of OB/GYN, Medical Director, Medical West Community Health Plan, 444 Montgomery St., Chicopee, MA 01020. Or call Joanne Alderman, M.P.H., Executive Assistant to the Medical Director, (413) 594-3111, collect.

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Interested candidates telephone **Dr. Martin Farber at (800) 222-0154 or (313) 593-7803** or send curriculum vitae to: **Martin Farber, M.D., Senior Vice President, Oakwood Health Services Corporation, 18101 Oakwood Blvd., P.O. Box 2500, Dearborn, MI 48123-2500.** Equal Opportunity Employer.

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SM

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Roger L. Wallace, D.O.
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Seeking BC/BE Reproductive Endocrinologist to run new REI sub-division within an 18 physician Department of Obstetrics and Gynecology.

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The Department of Obstetrics and Gynecology at the University of Pittsburgh is seeking a full-time faculty member to direct a program in Reproductive Immunology.

Applicants must be Board eligible/certified in Obstetrics and Gynecology and Maternal/Fetal Medicine and must have training in immunology.

Applicants should demonstrate interest and experience in basic and/or clinical research, undergraduate and graduate education and clinical practice.

Academic rank and salary will be commensurate with experience, training and achievements.

Send inquiry and curriculum vitae to:

Richard L. Sweet M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
University of Pittsburgh School of Medicine
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300 Halket Street
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OBSTETRICIAN/ GYNECOLOGIST

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If you like living in a great area, a diversity of patients and responsibilities, and a promising future, then direct your inquiries and curriculum vitae to:

Medical Center of Delaware
c/o Garrett H. C. Colmorgen, M.D.
Obstetrics and Gynecology
Maternal-Fetal Medicine
4755 Ogletown-Stanton Road
P.O. Box 6001
Newark, DE 19718

Chairman/Director of Education Department of Obstetrics and Gynecology

Harrisburg Hospital is seeking a person who is board certified in obstetrics and gynecology and has skills and experience as a clinician, teacher, and administrator. Affiliated with the Penn State University College of Medicine/ Hershey Medical Center in obstetrics and gynecology residency program. Harrisburg Hospital is fully accredited at 480 beds. Residency training is also provided in surgery, orthopedics, internal medicine, family medicine, and psychiatry. Harrisburg Hospital is a recognized leader in education and healthcare in Central Pennsylvania. Harrisburg is a city with a metropolitan area of approximately 500,000 people with excellent access to all major East Coast cities. Salary is negotiable based on abilities and experience. Resumes should be submitted to Jesse A. Weigel, M.D., Senior Vice President of Medical Affairs, Harrisburg Hospital, 111 South Front Street, Harrisburg, PA 17101.

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The Department of Obstetrics and Gynecology at the University of Pittsburgh is seeking a full-time faculty member as Director of Reproductive Genetics.

Applicants must be Board-Certified in Obstetrics and Gynecology and Human Genetics and demonstrate interest and experience in basic and/or clinical research, undergraduate and graduate education and clinical practice.

Academic rank and salary will be commensurate with experience, training and achievements.

Send inquiry and curriculum vitae to:

Richard L. Sweet, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
University of Pittsburgh School of Medicine
c/o Magee-Women's Hospital
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OBSTETRICIAN/ GYNECOLOGIST

Waterbury Hospital Health Center, a 400-bed teaching affiliate of Yale University School of Medicine, is currently seeking two Board Certified/Eligible Obstetrician Gynecologists to assume a thriving private practice. Limited time spent weekly at a satellite clinic also part of the position.

An outstanding opportunity for individuals desiring a well compensated position with no starting fees or overhead concerns. A two year contract with an exceptional salary and excellent benefits. Beautifully renovated hospital with 14 brand new SRM's. A charming New England community within easy access to Manhattan and Boston.

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The Terry Endowed Chair in Maternal and Child Health: The Emory University School of Public Health announces the recruitment of the School's first fully endowed chair. The chair is named in honor of Jules and Deen Terry, who were both advocates for the welfare of women and children. Dr. Jules Terry was the director of Family Health in Georgia and the Principal Investigator of the WHO Collaborating Center in Perinatal Care and Health Services Research in Maternal and Child Health. Academic appointment will be at the level of full professor, with tenure. The successful candidate should have an M.D. (preferably with a M.P.H.) or a Ph.D. in a relevant discipline with extensive research experience in maternal and child health. The candidate should have a scholarly research background with an interest in teaching and in developing an independent and/or collaborative research program. The position will commence on or before the 1992-93 academic year.

Please reply by January 15, 1992, with a letter of interest, a curriculum vitae, and the names of three references to J. Essien, M.D., Chair, Search Committee, Emory University School of Public Health, 1599 Clifton Road, N.E., Atlanta, Georgia 30329. Emory University is an Affirmative Action/Equal Opportunity Employer.

HAMAD MEDICAL CORPORATION
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Hamad Medical Corporation is a 1,065 bed referral facility with 4,100 staff and is the main health establishment in the country with its modern equipped facilities, is seeking for a well experienced person in the field of Lab. Andrology to supervise this Laboratory in the Corporation.

The required candidate should have the relevant qualifications and experience.

Salary is according to experience.

Send complete CVs, copies of certificates with contact telephone number / address and attach one recent photograph to:

Head of Recruitment Section
Personnel Department
Hamad Medical Corporation
P.O. Box: 3050
Doha – State of Qatar
(Ad ref. # RS/AD/NDMP-2/1991)

PERINATOLOGIST
ALASKA

Outstanding practice opportunity for 1-2 BC/BE perinatologists to develop perinatal services at the only Level III perinatal center in Anchorage. The center has an active perinatal transport program that draws from the whole state (11,000 births). The NICU is staffed by 6 in-house neonatologists. Components of the job include primary patient care, medical leadership for the perinatal center, maternal transport and outreach education to referral hospitals. Support staff for clinical research is also available.

Competitive salary and benefits program available through affiliation with neonatology group. Anchorage offers excellent recreational opportunities with the advantage of the amenities of a city.

Please send letter of interest with CV to Jack Jacob, M.D., Alaska Neonatology Associates, P.C., 3340 Providence Drive, Suite 366, Anchorage, Alaska 99508 or call (907) 563-3026.

FACULTY POSITIONS

OB/GYN

Beth Israel Hospital, a major Harvard Teaching Hospital, has faculty positions available for board eligible or board certified physicians in general OB/GYN. These positions provide an excellent opportunity for teaching and research. Special interest in the field of public health is recommended. Significant time will be available for either clinical/basic research or opportunity to receive a Public Health degree.

Salary and faculty appointment commensurate with curriculum vitae and bibliography.

Send curriculum vitae to:

Benjamin P. Sachs, M.D.
Obstetrician-Gynecologist-in-Chief
Beth Israel Hospital
330 Brookline Avenue
Boston, MA 02215
(617) 735-2266
Fax (617) 735-5011

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Beth Israel Hospital Boston

This established physician practice is offering an excellent opportunity for a BC/BE obstetrician. We're located in beautiful Southeastern New Hampshire, where you'll discover the ideal lifestyle. The location offers proximity to mountains, lakes and seacoast, plus a wealth of cultural attractions. It all adds up to a very healthy family environment.

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The Department of Obstetrics and Gynecology at the University of Pittsburgh is seeking a full-time faculty member to serve as Director for Research in Reproductive Sciences.

Applicants must be Board-Certified in Obstetrics and Gynecology and must have demonstrated experience in basic science laboratory research and administrative experience with collaborative research programs.

Academic rank and salary will be commensurate with experience, training and achievements.

Send inquiry and curriculum vitae to:

Richard L. Sweet, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
University of Pittsburgh School of Medicine
c/o Magee-Women's Hospital
300 Halket Street
Pittsburgh, PA 15213

The University of Pittsburgh is an equal opportunity, affirmative action employer.

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

The Department of Obstetrics and Gynecology at the University of Pittsburgh is seeking a full-time faculty member to serve as Director of House Staff Affairs and Medical Student Education.

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 76 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiform, Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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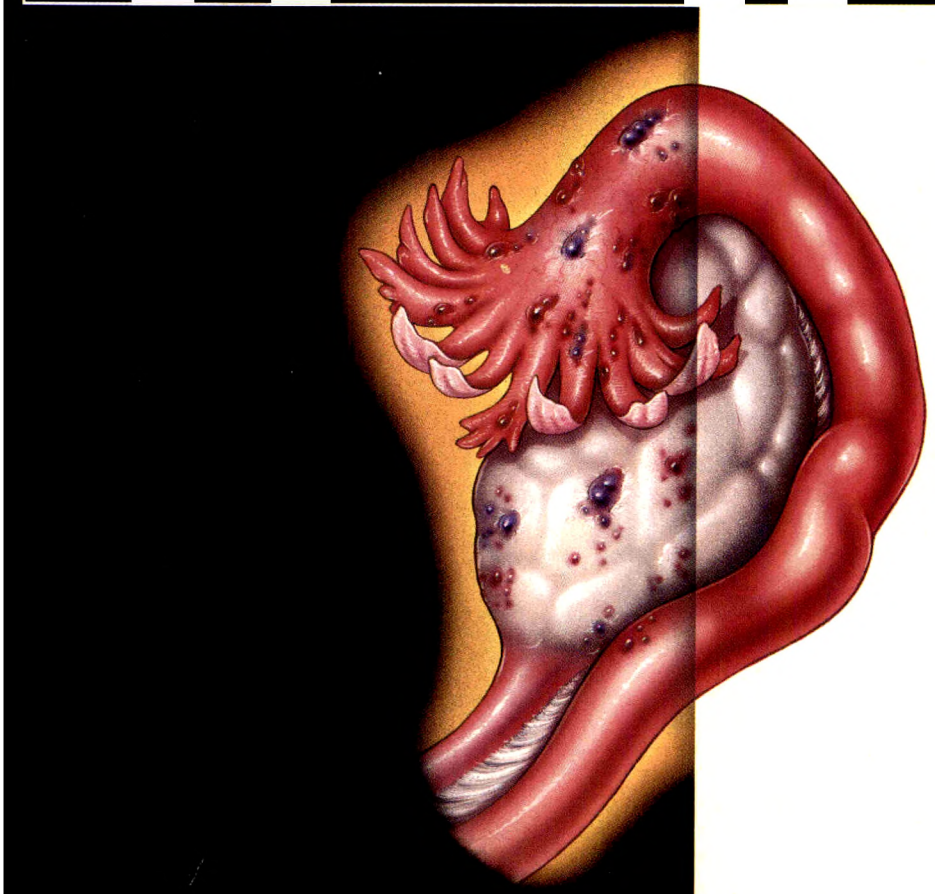
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*Pregnancy should be ruled out before therapy with SYNAREL is initiated, and patients should be advised to use a nonhormonal method of contraception when taking SYNAREL.
†There is a small loss in bone density with 6-month treatment, some of which may not be reversible. During one treatment period the bone loss should not be important. ‡Gonadotropin-releasing hormone analog. Please see brief summary of prescribing information on next page.



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BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve spontaneously or may require drug discontinuation or surgery.

Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the dose. Counsel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients. 3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician. 4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content. 6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption. 7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (± SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

SYNTEX

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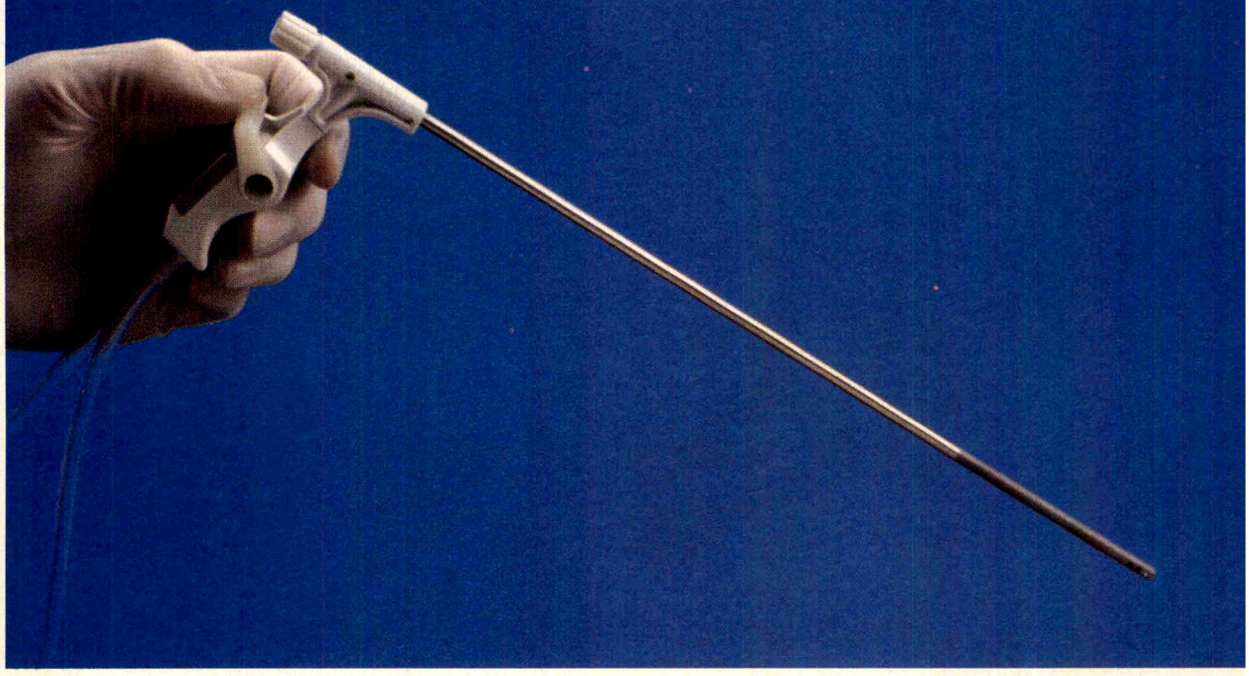
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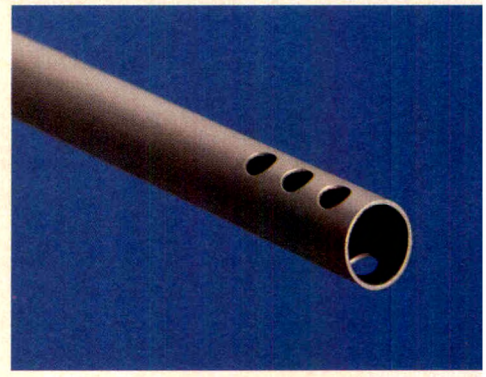
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A BRIEF SUMMARY FOLLOWS.

INDICATIONS AND USAGE
TREATMENT

CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Proteus mirabilis,* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K. pneumoniae*), *E. coli, Proteus mirabilis,* and *Serratia marcescens**.

Skin and Skin Structure Infections due to *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *Escherichia coli, Klebsiella pneumoniae, Peptococcus niger**, *Peptostreptococcus* species.

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *Streptococcus agalactiae, E. coli, Proteus mirabilis, Neisseria gonorrhoeae,* Bacteroides species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus niger* and *Peptostreptococcus* species).

Cefotetan, like other cephalosporins, has no activity against *Chlamydia trachomatis.* Therefore, when cephalosporins are used in the treatment of pelvic inflammatory disease, and *C. trachomatis* is one of the suspected pathogens, appropriate antichlamydial coverage should be added.

Intra-abdominal Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*) and *Clostridium* species*.

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic *in vitro* against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH CEFOTAN IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTETAN DISODIUM, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS-HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO CEFOTAN OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including cefotetan, and may range from mild to life-threatening. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *Clostridium difficile.*

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: The administration of CEFOTAN may result in a false-positive reaction for glucose in the urine using Clinitest®†, Benedict's solution, or Fehling's solution. It is recommended that glucose tests based on enzymatic glucose oxidase be used.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Pregnancy: Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy. Those appearing in italics have been reported during postmarketing experience.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700); *pseudomembranous colitis.* Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. (See WARNINGS.)

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300); *agranulocytosis, hemolytic anemia, leukopenia, thrombocytopenia,* and *prolonged prothrombin time.*

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700); *anaphylactic reactions.*

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

Miscellaneous: *Fever*

In addition to the adverse reactions listed above which have been observed in patients treated with cefotetan, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics: urticaria, pruritis, Stevens-Johnson syndrome, erythema multiforme, toxic epidermal necrolysis, vomiting, abdominal pain, colitis, superinfection, vaginitis including vaginal candidiasis, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, increased BUN, increased creatinine, elevated bilirubin, pancytopenia, and neutropenia.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see DOSAGE AND ADMINISTRATION and OVERDOSAGE). If seizures associated with drug therapy occur, the drug should be discontinued. Anticonvulsant therapy can be given if clinically indicated.

DOSAGE AND ADMINISTRATION
TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Skin & Skin Structure		
Mild-Moderate[a]	2 grams	2 g every 24 hours IV 1 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6 grams[b]	3 g every 12 hours IV

[a] *K. pneumoniae* skin and skin structure infections should be treated with 1 or 2 grams every 12 hours IV or IM.

[b] Maximum daily dosage should not exceed 6 grams.

If *C. trachomatis* is a suspected pathogen in gynecologic infections, appropriate antichlamydial coverage should be added, since cefotetan has no activity against this organism.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. See FULL PRESCRIBING INFORMATION for a complete description.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

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References: 1. Walker CK, Landers DV, Ohm-Smith MJ, et al. Comparison of cefotetan plus doxycycline with cefoxitin plus doxycycline in the inpatient treatment of acute salpingitis. *Sex Trans Dis.* 1991;18:119-123. **2.** McGregor JA, Gordon SF, Krotec J, Poindexter AN. Results of a randomized, multicenter, comparative trial of a single dose of cefotetan versus multiple doses of cefoxitin as prophylaxis in cesarean section. *Am J Obstet Gynecol.* 1988;158:701-706. **3.** Carver P, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986(suppl):11-14. **4.** Ohm-Smith MJ, Sweet RL, Hadley WK. In vitro activity of cefbuperazone and other antimicrobial agents against isolates from the female genital tract. *Antimicrob Agents Chemother.* 1985;27:958-960. **5.** Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. **6.** Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL. Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis.* 1986;4:359-363. **7.** Wexler HM, Finegold SM. In vitro activity of cefotetan compared with that of other antimicrobial agents against anaerobic bacteria. *Antimicrob Agents Chemother.* 1988;32:601-604. **8.** Hennessey TD. Cefotetan: a compilation of susceptibility data of clinical isolates. In: Lode H, Periti P, Strachan CJL, eds. *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections.* New York, NY: Churchill Livingstone; 1985:14-22. **9.** Centers for Disease Control. 1989 sexually transmitted diseases treatment guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

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A PILL WITH A PROGRAM

Only TRI-NORINYL starts your OC patients with The TRI-NORINYL Total Health Program.™

When you start your OC patient with The TRI-NORINYL Total Health Program sample package, you provide the benefits of oral contraception *plus* an exciting new patient-support program.

The TRI-NORINYL Total Health Program sample package contains one cycle of TRI-NORINYL; the attractive Wallette® Pill Dispenser; and a patient Q&A booklet—*plus* the video and instruction guide, *The ACOG Balanced Fitness Workout;* and program membership. The TRI-NORINYL Total Health Program provides a variety of fitness and health information to help women achieve healthier lifestyles.

As part of that goal, Syntex Laboratories is proud to be a sponsor of the American Medical Association's Women's Health Campaign.

For details about The TRI-NORINYL Total Health Program, please contact your Syntex Representative.



Serious as well as minor side effects have been reported following the use of oral contraceptives. These include thrombo-embolic disease.

Please see adjacent page for brief summary of prescribing information.

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TABLETS

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL)

Committed to the OB/GYN and women's healthcare

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA. 94304

TRI-NORINYL
TABLETS

(NORETHINDRONE AND ETHINYL ESTRADIOL)

ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS**, section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40–49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970s—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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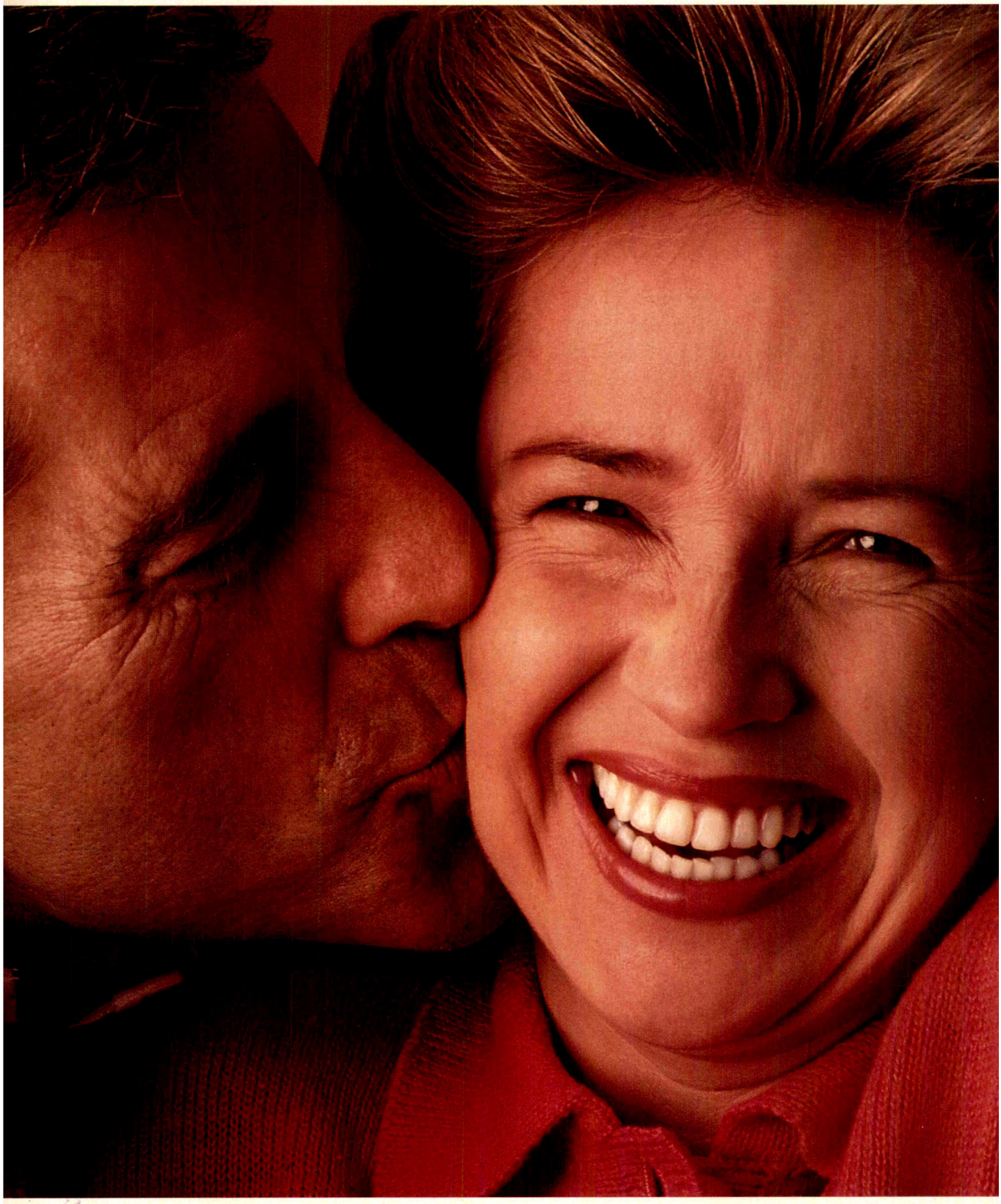
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Note: Estrogens have been reported to increase the risk of endometrial carcinoma in postmenopausal women.



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Please see brief summary of prescribing information on last page of this advertisement.

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.
There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risks, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy: changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

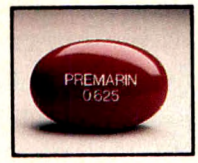
Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

PREMARIN®
(conjugated estrogens tablets)



The appearance of this tablet is a trademark of Wyeth-Ayerst Laboratories.

OSTEOPOROSIS
The only cure is prevention

Reference: 1. Weiss NS, Ure CL, Ballard JH, et al: Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. *N Engl J Med* 1980;303:1195-1198.

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American Journal of Obstetrics and Gynecology

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American Journal of Obstetrics and Gynecology

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INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors (N Engl J Med 1991;324:424-8). Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL.

Manuscript submission. Manuscripts should be submitted to one of the two Editors in Chief as follows:

1. **Dr. Zuspan**—manuscripts from the eastern and southeastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. J. Peter Van Dorsten, P.O. Box 34, MCV Station, Richmond, VA 23298.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

Author's suggested reviewers. When authors submit their manuscripts, they must provide the names and addresses of at least three reviewers for consideration by the Editors.

Copyright statement. The specified copyright statement that follows the Information for Authors in each issue of the JOURNAL must be completed, signed by all authors, and submitted with the manuscript. If not completed in full, it will be returned to the author for completion. The copyright statement and checklist form may be torn from the JOURNAL or photocopied for submission.

Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. *If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.*

Previous publication. If a report by the same author(s) or some of the same authors has been previously published in any medium that deals in any respect whatsoever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article(s) or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. Two copies of a new manuscript being prepared for possible publication also must be submitted. The author(s) should inform the Editor of the circumstances, similarities, and differences of the reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

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requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the *Material and Methods* section of the submitted manuscript. Reports of experiments on nonhuman animals or other species must *state in the* Material and Methods *section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed. The type of nonhuman animals or other species used in an investigation must be named in the title, abstract, key words, and *Material and Methods* section of the manuscript.

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General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of black-and-white glossy prints of illustrations are required. See "Color figures" and "Computer-generated figures" for special requirements. Manuscripts must be submitted in English.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, figure legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable, but honors degrees are not permitted); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, business and home telephone numbers, and fax number of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, business and home telephone numbers, and fax number of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. *At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).*

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Abstract page including key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Below the abstract, list 3 to 5 *key words or short phrases* for indexing purposes.

A structured abstract is required for certain types of articles and a *standard abstract format* is required for other types of articles; therefore the author(s) should select and prepare the appropriate abstract to be used in the submitted manuscript. This applies to both independently submitted and society manuscripts.

Structured abstract. A structured abstract, limited to 150 words, is to be used for regular articles, society articles, and Current Investigation articles; excluded are Clinical Opinion, Current Development, case report, and brief communication articles. The structured abstract is to contain the following major headings: *Objective(s); Study Design; Results;* and *Conclusion(s).* The *Objective(s)* reflects the purpose of the study, that is, the hypothesis that is being tested. The *Study Design* should include the setting for the study, the subjects (number and type), the treatment or intervention, and the type of statistical analysis. The *Results* include the outcome of the study and statistical significance if appropriate. The *Conclusion(s)* states the significance of the results.

Standard abstract. The standard abstract format is required for Clinical Opinion, Current Development, case report, and brief communication articles. The standard abstracts for Clinical Opinion and Current Development articles are to be 50 to 150 words and standard abstracts for case reports and brief communications are limited to 50 words. Even though the formal names of these four types of articles are not used, an author should determine his/her type of manuscript and prepare the appropriate standard abstract, regardless of whether it is to be submitted independently or as a part of a society's transactions.

Text. Do not hesitate to write your manuscript in the first person and active voice if they are more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through *Comment* section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

Council of Biology Editors Style Manual or the AMA's *Manual of Style.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify *Material and Methods*, *Results*, and *Comment*. Authors may wish to summarize their findings in a short paragraph at the end of the *Comment* section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to repeat the study) the plan, the patients, experimental animals or other species, material, and controls, the methods and procedures utilized, and the statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the *Comment* section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the *Results* section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number (25) is allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (N Engl J Med 1991;324:424-8). Journal titles should conform to abbreviations used in *Cumulated Index Medicus.*

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Dunnett C, Fischermann E, Quilligan EJ. Vaginal delivery following cesarean section: use of oxytocin augmentation and epidural anesthesia with internal tocodynamic and internal fetal monitoring. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: Ledger WJ. Dystocia and prolonged labor. In: Wilson JR, Carrington ER, eds. Obstetrics and gynecology. St. Louis: CV Mosby, 1987 vol 8:474-93.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. *This signed permission must accompany the manuscript when it is submitted to the Editor.* Published abstracts can be used as numbered references; however, reference to the complete published article is preferred.

Figures. The term *figure* includes all types of illustrations such as graphs, diagrams, photographs, flow charts, and line drawings. A reasonable number of figures will be reproduced without charge, but special arrangements must be made with the Editors for color figures at an additional charge to the author. The quoted charge is per page of color, and as many as eight color figures can be included on one page if the color balance is appropriate.

Figures must be cited consecutively in the text in Arabic numerals and identified with a gummed label on the back of the figure with author(s) name(s), title of article, figure number, and top marked clearly. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Do not use paper clips or mar the surface of figures in any way. Figures will be returned only on request by the author.

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These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

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—Guidelines for the care and use of *nonhuman* animals or *other species* approved by the institution have been followed as indicated in the *Material and Methods* section. *The species is named in the title, abstract, key words, and* Material and Methods *section.*
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed in English double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
—All pages are numbered in the following order: title page, condensation, structured or standard abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
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—Names and addresses of three suggested reviewers are enclosed.

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—These elements are given in the following sequence, and are typed double-spaced.
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—The name(s) of the institution(s) section(s), division(s), department(s) in which the study was performed is provided and the institutional affiliation(s) of the author(s) at the time of the study is indicated.
—Acknowledgment of financial support is cited.
—Name, address, business and home telephone numbers, and fax number of author to whom requests for reprints are to be sent are included.
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Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract and key words or short phrases

—The abstract (structured or standard format) is typed double-spaced with required margins on page 3 headed by the title and author(s) name(s). Beneath the abstract 3 to 5 key words or short phrases are typed.
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—Three sets of unmounted glossy prints for *black-and-white* figures are submitted, properly numbered, labeled, and *top indicated* on the back of each print.

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—Are provided for each figure.

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AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

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NORPLANT® SYSTEM

levonorgestrel implants

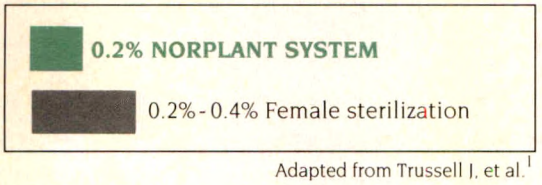


Six reasons why you can feel comfortable recommending it to your patients

Effectiveness comparable to tubal ligation

Although no contraceptive is 100% effective, the NORPLANT SYSTEM is one of the most effective forms of birth control ever developed. The average annual gross pregnancy rate over a five-year period is less than 1%.

Lowest expected and typical failure rates with NORPLANT SYSTEM vs. sterilization during first-year use[1]



Adapted from Trussell J, et al.[1]

Long-term convenience... estrogen-free

NORPLANT SYSTEM, a progestin-only contraceptive, is an excellent option for women desiring a long-term method. Its effectiveness lasts for five years and is not dependent on compliance.

Reversible, with prompt return to previous level of fertility

NORPLANT SYSTEM is reversible at any time; the contraceptive effect ceases when the capsules are removed. In one study, 40% of women wanting to conceive did so within 3 months of removal, 63% by 6 months, 76% by 12 months, and 90% by 24 months.[2]



High patient acceptance despite menstrual pattern changes

NORPLANT SYSTEM causes menstrual irregularities (the most common complaint),* which occur in many women during first-year use and diminish over time. Only 9.1% of patients discontinued during first-year use due to these changes.[3†]

Women who are thoroughly informed of potential menstrual changes and side effects are more tolerant of them when they occur.[4]

Well-received, well-liked by American women

One study of 140 users revealed that 94% were satisfied with NORPLANT SYSTEM, 91% recommended it to their friends, and 74% wanted to use it again.[5]

A 10- to 15-minute office procedure

A local anesthetic and a small 2 mm incision...little or no discomfort during insertion...proper placement should not leave a noticeable scar...in most cases sutures are not needed.

*Serious as well as minor side effects may occur (see prescribing information).

†Based on multicenter trials of 2,470 women.

Please see brief summary of prescribing information on adjacent page.

NORPLANT® SYSTEM
levonorgestrel implants

Lasts 5 years...yet is reversible

NORPLANT® SYSTEM

levonorgestrel implants

Lasts five years…yet is reversible

BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See **Warnings** section and Full Prescribing Information.

NURSING MOTHERS: See **Warnings** section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

CI 4064-1 12/10/90

References: 1. Trussell J, Hatcher RA, Cates W, et al: *Stud Fam Plann* 1990;21:51-55. **2.** Sivin I, Diaz S, Holma P, et al: *Stud Fam Plann* 1983;14:184-191. **3.** Data on file, Wyeth-Ayerst Laboratories. **4.** Alvarez-Sanchez F, Brache V, Faundes A: *Stud Fam Plann* 1988;19:118-121. **5.** Darney PD, Klaisle CM, Tanner S, et al: *Curr Probl Obstet Gynecol Fertil* 1990;13:89-125.

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CLINICAL SECTION

Clinical Articles

Low-dosage micronized 17β-estradiol prevents bone loss in postmenopausal women

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Oakland and San Francisco, California, and Waltham, Massachusetts

With the use of a double-blind, randomized, dose-ranging design, we tested during an 18-month period the degree of protection against postmenopausal bone loss afforded by micronized 17β-estradiol in dosages of 0.5, 1.0, and 2.0 mg. All subjects received supplementation to ensure a minimum of 1500 mg calcium daily. Fifty-one subjects completed at least 1 year of follow-up bone density measurements by quantitative computed tomography and by single- and dual-photon absorptiometry. In the placebo group spinal trabecular bone density decreased 4.9% annually ($p < 0.001$), whereas in those taking micronized 17β-estradiol bone density tended to increase (annual increases of 0.3% in the 0.5 mg micronized 17β-estradiol group, 1.8% in the 1.0 mg micronized 17β-estradiol group, and 2.5% in the 2.0 mg micronized 17β-estradiol group). After completing the double-blind phase, 41 subjects completed an additional 18 months of follow-up while taking 1.0 mg micronized 17β-estradiol. During this time one third of the subjects were randomly assigned to discontinue calcium supplements. Among those who previously received placebo, trabecular bone density increased 4.3% annually, whereas among those who had used micronized 17β-estradiol, trabecular bone density response was inversely related to the dosage previously used. Additionally and independently, the level of calcium intake showed a statistically significant correlation with the change in spinal trabecular bone density ($r = 0.37$, $p = 0.02$). We conclude that micronized 17β-estradiol has a continuous skeletal dose-response effect in the range of 0.5 to 2.0 mg and that calcium intake positively modifies the skeletal response to 1.0 mg micronized 17β-estradiol. (AM J OBSTET GYNECOL 1992;166:479-88.)

Key words: Absorptiometry, photon; bone density; calcium, dietary; estradiol; osteoporosis, postmenopausal

Ample evidence exists that estrogen deficiency causes bone loss in women. Estrogen replacement therapy can prevent or retard these losses, and long-term use reduces by 50% the risk of osteoporotic fractures.[1,2] Skeletal protection has been shown for various estrogen dosages: Minimum dosages of some estrogens have been determined from an uncontrolled observational study[3] and from double-blind, randomized, controlled clinical trials.[4-7] In an observational study[8] we found that most women could be protected against bone loss by taking half the customary dosage of conjugated estrogen plus a 1500 mg calcium intake. Several criticisms were leveled at that study: It did not use randomization or placebo controls, a low level of endogenous estrogen production could have persisted in the recently menopausal subjects, and two thirds of the women were also given a progestogen that could have enhanced estrogen's skeletal protection.

To assess the minimum effective dosage of micronized 17β-estradiol and to investigate the possible synergism between low-dosage estrogen and calcium, we designed a two-phase clinical trial, each phase lasting 18 months. The first was a double-blind, placebo-con-

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6/1/32024

trolled, dose-ranging study. Because previously published studies of micronized 17β-estradiol showed that 2.0 mg produced an increase in bone mass but 1.0 mg did not fully prevent bone loss,[7] we chose a dosage range of 0.5 to 2.0 mg. These dosages appear to be biologically equivalent to 0.3 to 1.25 mg of conjugated estrogens.[9] Calcium supplementation, sufficient to raise the daily intake to 1500 mg, was given to all participants, but no progestogen was used. Surgically castrated women and spontaneously menopausal women were included. A second phase was designed to evaluate the possible synergism between calcium and low-dosage micronized 17β-estradiol. All subjects completing the first phase were given 1.0 mg micronized 17β-estradiol, and one third were randomly assigned to discontinue calcium supplements.

Material and methods

Subjects. Postmenopausal, predominantly white women aged 40 to 58 years were solicited by advertisement and by physician referral from among San Francisco Kaiser Permanente Medical Center members. The study protocol was approved by the Institutional Review Boards of the Kaiser Permanente Medical Care Program, Northern California Region, and the University of California, San Francisco, Medical Center. Menopause was confirmed by surgical report of bilateral oophorectomy or by absence of menses for ≥6 months. Estrogen deficiency was confirmed biochemically by the finding of a serum follicle-stimulating hormone level >40 U/L. At the start of the study, subjects were required to be within 5 years of menopause; they were stratified by years since menopause (<2.5 and ≥2.5 years). All women were ambulatory and healthy. Criteria for exclusion included presence of diseases or conditions known to affect skeletal health, including thyroid disorders, parathyroid disorders, any other disorders of calcium homeostasis, and use of anticonvulsants or glucocorticoids. Specifically excluded were women with evidence of renal, hepatic, cardiac, or malignant diseases. Body weight was required to be within 20% of ideal for height, as determined by Metropolitan Life Insurance Company tables.

Treatment

Phase 1. After informed consent was obtained, subjects were randomly assigned to placebo or identical-appearing active treatment with either 0.5, 1.0, or 2.0 mg of micronized 17β-estradiol (Estrace, Mead Johnson Laboratories, Div. of Bristol-Myers Co., Evansville, Ind.). Study pills were administered in a cyclic regimen on 23 of 28 days for 18 months. Pills returned unused at each 3-month visit were counted, and a new supply of pills was given. Calcium carbonate supplements, in the form of oyster shell calcium (Os-Cal, Marion Laboratories, Kansas City, Mo.), were given to raise the daily calcium intake above 1500 mg. In most cases 750 or 1000 mg of elemental calcium was required, but 29% of subjects required only 500 mg. The supplement was administered with meals, usually in two divided dosages.

Phase 2. On completing phase 1, subjects were not informed of their previous treatment assignment. They were counseled by an independent endocrinologist who was made aware of their previous treatment and bone density tests. This physician recommended participation in phase 2 unless previous use of 1.0 mg micronized 17β-estradiol had not protected that person against bone loss. After informed consent was obtained, subjects were enrolled in an open study in which all received 1.0 mg micronized 17β-estradiol for 25 days per month. Because we wanted to avoid subjecting these women to further risk of endometrial stimulation from prolonged exposure to unopposed estrogen, women with an intact uterus were also given medroxyprogesterone acetate, 10 mg daily, from day 13 to day 25 of each month. All subjects were advised to continue their usual diet, but, on the basis of a random number selection, one third of the subjects were instructed to discontinue calcium supplementation.

Bone mineral measurements. The primary efficacy assessment was spinal trabecular bone mass measured by quantitative computed tomography at the University of California Radiology Department. With a scanner (GE 9800, General Electric, Milwaukee), at 80 kV(p), this method obtains slices 10 mm thick through the midplane of vertebral bodies T-12 through L-3 and compares the density of an elliptic region of interest within each vertebra with a simultaneously scanned phantom containing different solutions of potassium phosphate. Results are expressed as trabecular bone density, in milligram per cubic centimeter potassium phosphate equivalents. Short-term precision in vivo was 1.5%,[10] whereas long-term precision, in vitro, determined on a phantom simulating a human torso, was 1.6%.[11] An analysis of statistical power estimated that sample sizes of nine per group would have a 90% chance ($p = 0.05$) of detecting a 4% change in spinal trabecular bone density.

In addition, two other density measurements were obtained. Spinal dual-photon absorptiometry was done with a rectilinear scanner (model 2600, Norland Corp., Fort Atkinson, Wis.) with a spatial resolution of 3 mm and long-term precision of 2.5%.[12] Results are expressed as bone mineral density in grams per square centimeter. In vitro phantom scans helped correct for longitudinal drifts associated with changes in gadolinium source and analytic software.[12] Radial single-photon absorptiometry was done with a Norland 278 digital bone densitometer. The radius is almost entirely cortical bone at the junction of the proximal two thirds and the distal one-third site used for measurement. The

Table I. Baseline characteristics by treatment in 63 women enrolled in micronized estradiol trial

	Placebo	*Micronized estradiol*		
		0.5 mg	*1.0 mg*	*2.0 mg*
No. of subjects	16	15	16	16
Age (yr, mean ± SD)	52.8 ± 4.5	53.1 ± 3.8	52.8 ± 2.9	50.0 ± 4.0
<2.5 yr since menopause (%)	56.3	46.7	62.5	56.3
Time since menopause (mo, mean ± SD)	33.7 ± 19.9	35.1 ± 19.2	31.1 ± 14.4	27.3 ± 19.9
Caucasian (%)	81.3	66.7	93.8	56.3
Height (in, mean ± SD)	63.7 ± 2.8	63.6 ± 3.0	64.3 ± 2.7	64.1 ± 2.6
Weight (lb, mean ± SD)	139.4 ± 24.1	139.9 ± 24.1	141.1 ± 19.3	129.9 ± 18.2
Dietary calcium (mg/day, mean ± SD)	745 ± 260	818 ± 202	871 ± 393	765 ± 227
Currently smoking (%)	31.1	20.0	18.8	18.8
Regular exercise or sports (%)	37.5	46.7	25.0	37.5
Kupperman index (mean ± SD)	15.5 ± 10.8	14.2 ± 10.7	13.0 ± 10.0	12.9 ± 7.8
Spinal trabecular bone density (mg/cm^3, mean ± SD)	116 ± 32.2	120.0 ± 36.0	115.2 ± 23.0	111.1 ± 17.4
Spinal bone mineral density (gm/cm^2, mean ± SD)	0.94 ± 0.15	0.92 ± 0.22	0.98 ± 0.15	0.94 ± 0.14
Radial bone mineral density (gm/cm^2, mean ± SD)	0.68 ± 0.07	0.68 ± 0.09	0.71 ± 0.04	0.67 ± 0.06

nondominant arm was measured three times; the arm was removed from the scanner and repositioned, and measurements were repeated three times; the subject was again removed, repositioned, and measured three more times. With this procedure, the average of nine scans per sitting yielded a long-term precision of 2.0%. Results are expressed as bone mineral density in grams per square centimeter.

Bone density measurements were done every 6 months during the first phase of the study. For each study participant the slope, intercept (at baseline), and annual percentage change (slope divided by intercept) were determined from all available measurements with the least-squares regression formula. To be considered evaluable for bone density outcome analysis, subjects were required to have at least 1 year of follow-up and three bone density studies. Bone density measurements were done once again after 18 months of phase 2, and the annual percentage change was calculated from the difference between the final measurements of phase 1 and those of phase 2.

Other measurements. A food frequency dietary history, based on consumption of 30 calcium-containing foods, was obtained at baseline and at each 3-month visit. A daily average was calculated from the monthly estimate after the addition of 100 mg of calcium per day from noncoded sources. Menopausal symptoms were recorded at each visit, and a total score was calculated from the severity of 11 menopausal symptoms, according to the weighting system developed by Kupperman et al.[13]; the maximum score on the Kupperman index is 51.

When possible, endometrial biopsies were done at the beginning and end of phase 1. Unexpected uterine bleeding was also an indication for biopsy. Tissue samples were analyzed by a pathologist who was unaware of treatment status.

Subjects continued in the trial unless they voluntarily withdrew, were removed from study because of an adverse event, or, after 12 months, had lost spinal trabecular bone density at an annual rate >8%.

Results

Phase 1. Baseline characteristics of 63 women enrolled in the study are displayed in Table I. Randomization and stratification by menopausal age succeeded in providing similar bone mineral densities among treatment groups. The 29 subjects who were 2.5 to 5.0 years postmenopausal were 2.7 years older than the 34 subjects <2.5 years beyond menopause. The later postmenopausal group had 10.2% lower spinal trabecular bone density (109.6 ± 32.8 vs 122.1 ± 21.3 mg/mm^3; $p < 0.05$), 9.1% lower spinal bone mineral density (0.900 ± 0.180 vs 0.990 ± 0.15 gm/cm^2; $p = 0.01$), and 4.3% lower radial bone mineral density (0.670 ± 0.080 vs 0.700 ± 0.050 gm/cm^2; not statistically significant).

Compliance was excellent. Only one subject used <75% of the required number of pills. Overall, 41 subjects (65%) completed 18 months of follow-up. Eight left the study before any follow-up was done. Four additional subjects, all assigned to the active drug group, discontinued study after the first 6-month follow-up measurements for personal reasons unrelated to treatment or bone density changes. In all these cases bone density at 6 months was higher than that at baseline.

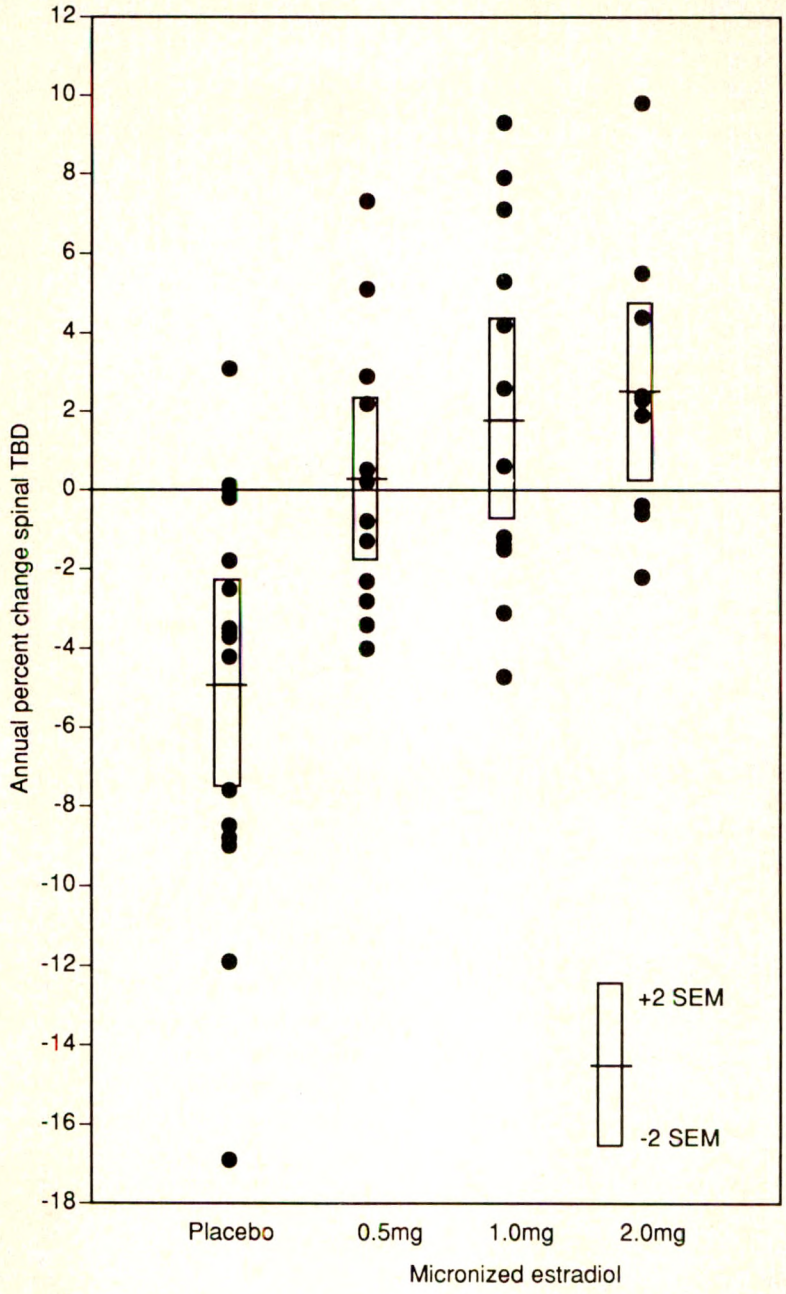


Fig. 1. Changes in mean (±2 SEM) annual percentage and each subject's spinal trabecular bone density *(TBD)* in 46 postmenopausal women given micronized estradiol (phase 1). All subjects received calcium supplements.

On the basis of the predetermined rule requiring a minimum of three bone measurements, data from these 12 subjects have been excluded from the outcome analysis. At 1 year of follow-up, five subjects taking placebo were removed from the trial because they had lost >8% of spinal trabecular bone density. Five additional subjects withdrew between the 12- and 18-month visits, two because of troublesome vaginal bleeding and three for reasons unrelated to treatment effects. Some adverse reaction, of at least moderate degree, was recorded for 37% of placebo users and for 33% of 0.5 mg, 37% of 1.0 mg, and 56% of 2.0 mg micronized 17β-estradiol users.

Placebo users had a statistically significant decrease in spinal trabecular bone density; the mean annual loss was 4.9% (95% confidence interval 2.4% to 7.5%). The changes in spinal trabecular bone density in all three groups treated with micronized 17β-estradiol were each statistically significantly different from the changes in the placebo group (analysis of variance, $p < 0.001$, vs

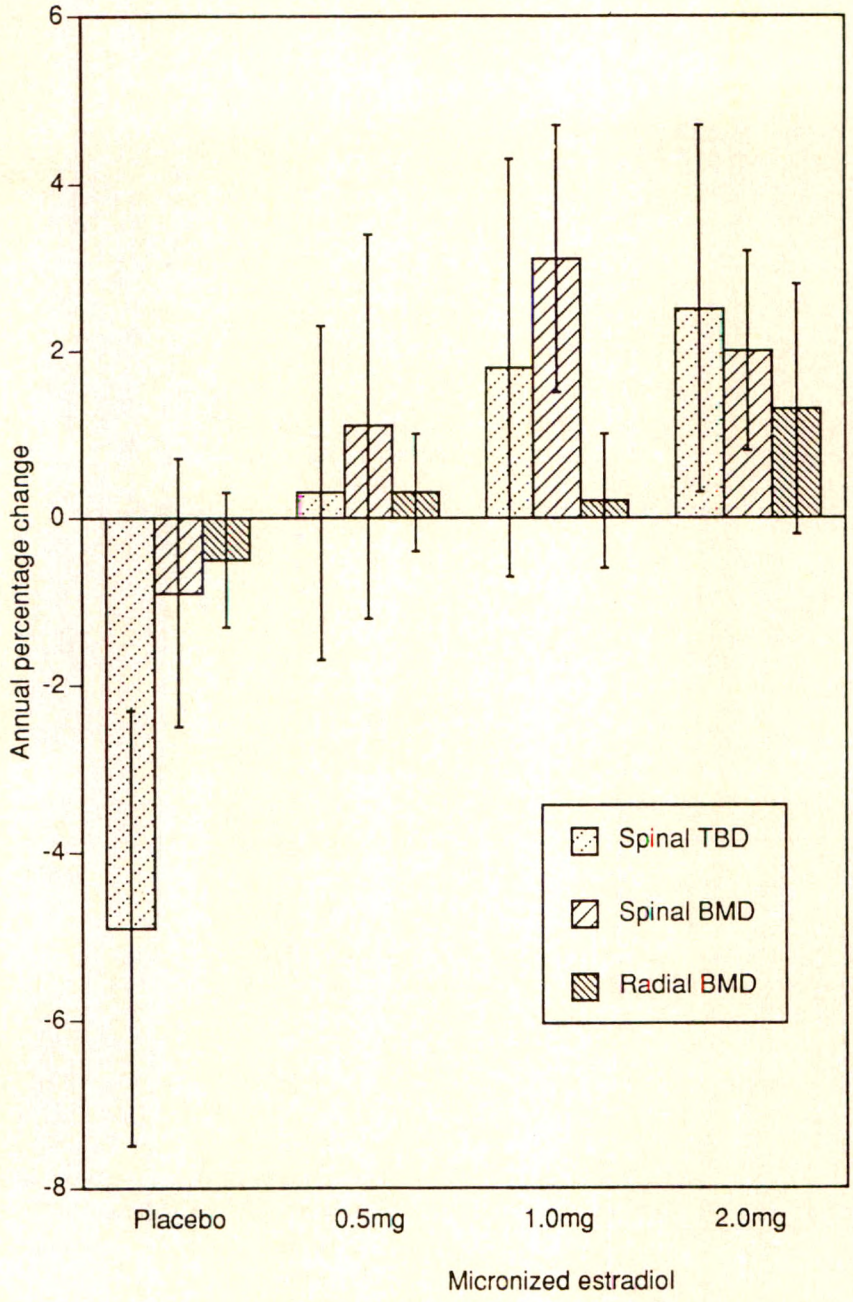


Fig. 2. Mean (±2 SEM) annual percentage change in spinal trabecular bone density *(TBD)*, spinal bone mineral density *(BMD)*, and radial bone mineral density in 46 postmenopausal women given micronized estradiol (phase 1). All subjects received calcium supplements.

placebo; Fig. 1). In the placebo group changes from baseline spinal bone mineral density (−0.9%) and radial bone mineral density (−0.5%) were not statistically significant. Changes in spinal and radial bone mineral density, although not statistically significant, tended to parallel the changes in spinal trabecular bone density (Fig. 2). The three bone measurements were statistically significantly correlated: spinal trabecular bone density versus spinal bone mineral density, $r = 0.58$, $p = 0.0001$; spinal trabecular bone density versus radial bone mineral density, $r = 0.28$, $p < 0.05$; spinal versus radial bone mineral density, $r = 0.28$, $p < 0.05$. Although we found no statistically significant differences in any bone parameter among the different micronized 17β-estradiol dosage groups, women assigned 1.0 and 2.0 mg dosages tended to have greater increases in spinal trabecular bone density than women assigned the 0.5 mg dosage (Fig. 2). When we compared women in all active treatment groups with women taking placebo, both spinal and radial bone mineral density

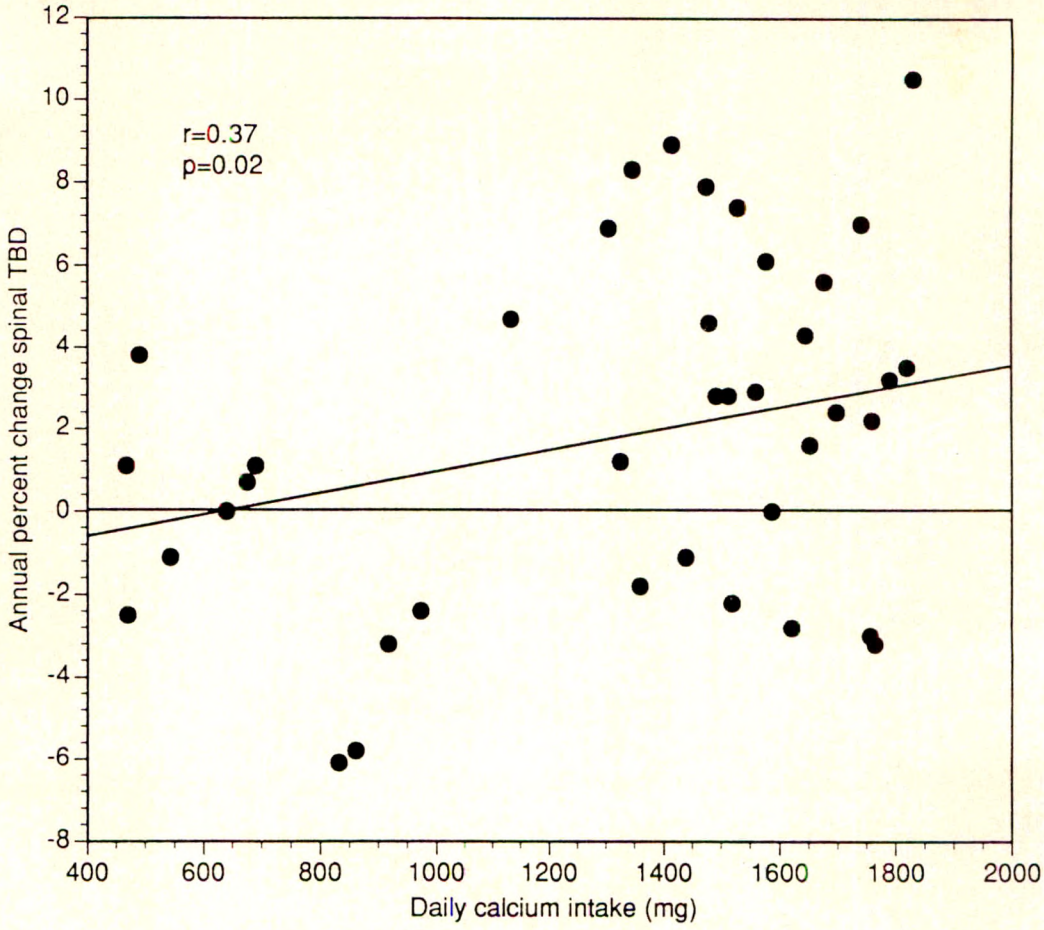


Fig. 3. Plot of annual percentage change in spinal trabecular bone density *(TBD)* in 41 postmenopausal women given 1.0 mg micronized estradiol by level of calcium intake (phase 2).

Table II. Endometrial biopsy results by treatment group

		Micronized estradiol		
	Placebo	*0.5 mg*	*1.0 mg*	*2.0 mg*
No. of subjects	14	8	11	9
Refused biopsy	6	1	4	0
Biopsy could not be performed	2	1	0	0
Atrophic endometrium	4	1	2	0
Proliferative endometrium	2	4	3	7
Hyperplasia of endometrium				
Cystic	0	0	0	1
Simple (with no atypia)	0	1	1	1
Complex (with atypia)	0	0	1	0

showed statistically significant differences. Smoking habit, type of menopause (spontaneous versus surgical), and recency of menopause did not statistically significantly influence changes in bone density.

Of the entire cohort, 17.5% had previous removal of ovaries and uterus. Of the women still at risk for gynecologic complications, no uterine bleeding was observed among 14 taking placebo or among 11 taking 0.5 mg micronized 17β-estradiol. Of 13 women taking 1.0 mg micronized 17β-estradiol, three experienced light withdrawal bleeding and one had unexpected troublesome bleeding. One of 14 women using 2.0 micronized 17β-estradiol experienced light withdrawal bleeding, and three others had unexpected troublesome bleeding. The results of endometrial sampling done at the end of phase 1 are displayed in Table II; hyperplasia was found in five of 23 (22%) biopsy specimens obtained from women taking micronized 17β-estradiol. Of these five, one had no bleeding, one had light, regular withdrawal bleeding, and the remaining three had irregular and heavy bleeding. The mean menopause symptom score during treatment was unchanged from baseline in women taking placebo (14.3 ± 7.1) but was statistically significantly reduced to 9.2 ± 10.1 ($p < 0.05$) in women taking 0.5 mg mi-

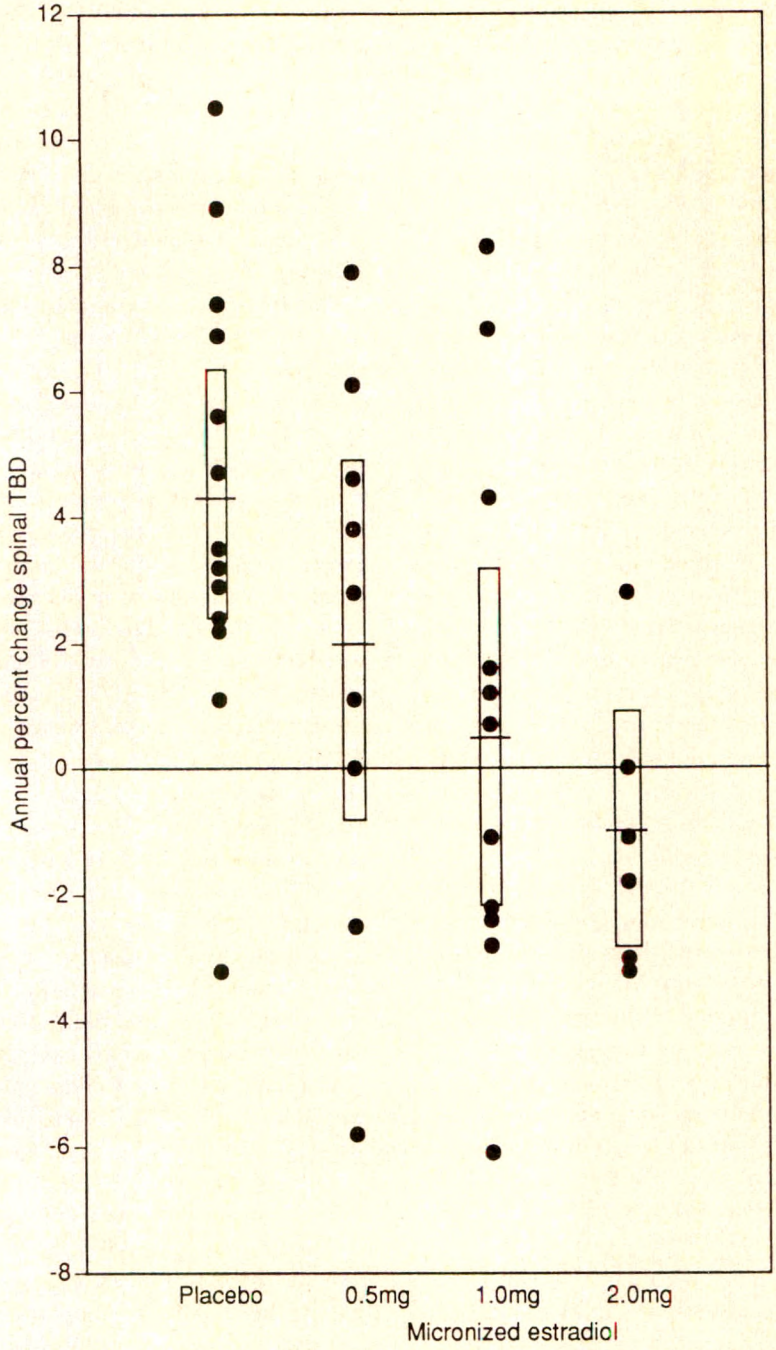


Fig. 4. Changes in mean (±2 SEM) annual percentage and each subject's spinal trabecular bone density *(TBD)* in 41 postmenopausal women given 1.0 mg micronized estradiol versus prior treatment (phase 2).

cronized 17β-estradiol, to 7.3 ± 13.0 ($p < 0.05$) in women taking 1.0 mg micronized 17β-estradiol, and to 7.5 ± 5.9 ($p < 0.01$) in women taking 2.0 mg micronized 17β-estradiol.

Phase 2. All subjects invited to continue agreed to comply with the drug and dietary instructions, and 41 completed the additional 18-month follow-up measurements. Women who were instructed to stop calcium supplements had an average daily dietary calcium intake similar to that which they received in phase 1 (830 ± 337 vs 804 ± 263 mg). Similarly, women assigned to continue calcium supplements did not statistically significantly alter their dietary calcium intake (826 ± 316 vs 787 ± 257 mg). Women who left this

phase of the trial (9.8%) did so for personal reasons, and none experienced serious adverse reactions. Of the 37 women with an intact uterus, seven (18.9%) had no withdrawal bleeding, two (5.4%) experienced fewer than three menses a year, and 28 (75.7%) had regular monthly withdrawal bleeding.

During the 18 months of extended treatment with 1.0 mg micronized 17β-estradiol, the annual change in spinal trabecular bone density was +2.0% (95% confidence interval 0.7% to 3.3%). We found a statistically significant positive correlation between total calcium intake and change in spinal trabecular bone density ($r = 0.37$, $p = 0.02$; Fig. 3). Previous treatment was a statistically significant independent predictor of the spinal trabecular bone density response to 1.0 mg micronized 17β-estradiol (Fig. 4). The greatest increases were observed among women previously assigned to placebo, with an average annual gain of 4.3% (95% confidence interval +2.3% to +6.3%). Women previously assigned to 0.5 mg micronized 17β-estradiol gained 2.0% (95% confidence interval −0.8% to +4.8%), women continuing on a regimen of 1.0 mg gained 0.5% (95% confidence interval −2.1% to +3.1%), and women whose dosage was reduced from 2.0 to 1.0 mg lost 1.0% (−2.8% to +0.8%). Changes in spinal trabecular bone density did not have a statistically significant correlation with smoking habit, type or recency of menopause, use of progestogen, previous rate of change in spinal trabecular bone density, or absolute level of spinal trabecular bone density. For the entire cohort, mean spinal bone mineral density increased 2.9% (95% confidence interval +1.7% to +4.1%), but we found no statistically significant correlations between changes in this measurement and previous dosage, calcium intake, or other clinical variables. We observed no statistically significant changes for radial bone mineral density (+0.3%), and this measurement did not correlate with any clinical variables.

Comment

Estrogen-deficient women lose mineral at all skeletal sites, but the greatest losses are in the trabecular bone of the vertebrae. Because of its high surface-to-volume ratio and its more rapid turnover, trabecular bone typically responds two to three times as much as cortical bone. Spinal trabecular bone density, with measurement of only the trabecular component of the vertebra, usually shows annual losses of 5% in women who have recently become estrogen deficient. Initiating estrogen therapy in women who have recently lost ovarian function typically increases spinal trabecular bone density about 5% in the first year and thereafter maintains it at a relatively constant level.[8] Both phases of the current study show this effect, which is probably related to a temporary imbalance between bone resorption and formation. Estrogen rapidly reduces bone resorption but allows the continuing repair and mineralization of previously active resorption sites (bone metabolic units). The skeletal response to estrogen (and other agents that reduce bone resorption) depends, in large part, on the degree of skeletal activation when treatment is begun. Gains in bone mass would be expected in women with a high turnover rate and a large skeletal remodeling space.

The first phase of our study lacked sufficient power to detect differences among women taking different dosages of micronized 17β-estradiol. We might have concluded, at the end of the first 18 months, that maximum skeletal protection could be achieved by 0.5 mg of micronized 17β-estradiol and that higher dosages did not augment this protection. However, in the second phase, when women previously taking 0.5 mg were given 1.0 mg, they appeared to show a further increase in spinal trabecular bone density. Conversely, women taking 2.0 mg appeared to lose bone when the dosage was reduced. On the basis of these observations, we conclude that there is a continuous dose-response effect in the range of dosages tested.

The first phase of our study was designed to assess the effects of estrogen apart from the usually coprescribed progestogen. In the second phase we were unable to show that medroxyprogesterone, 10 mg daily, given for 12 days per month, had any skeletal effects, although our power to detect this was limited. Although some have indicated that certain progestogens, particularly some 19-norsteroid derivatives, may prevent bone loss,[14] this effect has not been found for other 19-norsteroid progestogens or for medroxyprogesterone acetate, even in daily dosages of 20 mg.[15] Although the addition of a progestogen is not necessary for bone protection, it is unwise and impractical to give unopposed estrogens to women who still have an intact uterus. The incidence of troublesome bleeding and endometrial hyperplasia found when unopposed estrogen was used in our study is consistent with that of many other reports of unopposed estrogen use.

Most studies of women in the immediate postmenopausal years indicate that calcium has little or no effect.[8, 16, 17] Riis et al.[18] suggested that calcium, 2000 mg/day, could partially retard loss of total body calcium and cortical bone in the proximal radius, but it could not protect against spinal or distal radial mineral loss. Women who are several years beyond menopause[17, 19] or are elderly[20, 21] may derive some benefit from calcium. Certainly, very low calcium intakes should be avoided; they are associated with higher rates of bone loss[22] and increased risk of hip fracture.[23] In a study of 87 Danish women, Christensen et al.[7] tested micronized 17β-estradiol dosages of 1.0, 2.0, and 4.0 mg. These women, whose dietary calcium intake was probably

higher than that of women in the United States, were also supplemented with 500 mg of calcium. Bone mass as determined by radial single-photon absorptiometry decreased 2% in the placebo group, was unchanged in the low-dosage group, and was increased 1.5% and 2.0% in the medium- and high-dosage groups, respectively. In a nonrandomized, open study, Quigley et al.[1] compared 1.0 mg micronized 17β-estradiol or 0.625 mg conjugated estrogen with a regimen of no hormone treatment in 397 postmenopausal women aged 51 to 80 years. Subjects were asked to take 1000 mg of calcium daily if they were taking estrogen and 1500 mg if they declined estrogen treatment. Progestogens were not used. In each subject radial bone mineral density measurements were made initially and at the end of 3 years. Bone loss in both estrogen-treated groups was significantly less than in women using calcium alone, and the amount of protection provided by 0.625 mg conjugated estrogens was comparable with that provided by 1.0 mg micronized 17β-estradiol. Although the investigators in that study did not test lower dosages of either estrogen preparation and did not assess the axial skeleton, their conclusions are consistent with those of our study.

Protective effects of estrogen replacement therapy on bone mass have been shown in a randomized, prospective study with a mean 9-year follow-up.[24] Epidemiologic investigations have found greater bone mass among long-term estrogen users than among nonusers.[2, 25] Lindsay and Tohme[25] reported that spinal and femoral bone mass, measured by dual-photon absorptiometry, was about 20% greater in women who had used estrogen for 10 years. Spinal quantitative computed tomography measurements indicate even greater salutary effects; women using estrogen for an average of 17 years had about 50% higher levels than did nonusers.[2]

Can these same benefits be achieved with half as much estrogen? A synergism between estrogen and calcium is supported by several lines of evidence. An additive effect of endogenous serum estrone and calcium was observed in an epidemiologic study of radial bone density in 174 postmenopausal women.[26] In a prospective trial premenopausal women, mean age 37 years, were randomly assigned to consume more dairy products or to continue their usual diet.[27] In women assigned enhanced diets, with the average daily calcium intake increased by 610 mg, bone mineral density was maintained in a 3-year period, whereas bone was lost in control subjects. During the study some degree of ovarian insufficiency might have developed in these women. Just as the osteotrophic effects of suboptimal endogenous estradiol production may be enhanced by high calcium intake, suboptimal estrogen replacement therapy also may be enhanced. We previously reported that combining 0.3 mg conjugated estrogens with 1500 mg calcium was as effective as 0.6 mg of conjugated estrogens with no added calcium.[8] Extending these observations from 2 to 3 years yielded similar conclusions, although spinal trabecular bone density decreased between the second and third years, radial bone mineral density and metacarpal cortical thickness were well maintained,[28] and the 0.3 mg dosage with calcium appeared indistinguishable from the 0.6 mg dosage with no calcium. Our study lacked sufficient power to conclusively judge the comparative efficacies of these two estrogen regimens. Gallagher et al.[15] replicated these findings in a 2-year randomized study, which concluded that calcium alone, 1000 mg daily, was ineffective in preventing bone loss measured by quantitative computed tomography, dual-photon absorptiometry, single-photon absorptiometry, and total body calcium level, but 0.3 mg conjugated estrogens combined with 10 mg medroxyprogesterone acetate and a total intake of 1000 mg calcium was as effective as 0.625 mg conjugated estrogens alone. Genant et al.,[5] testing various dosages of piperazine estrone sulfate combined with 1000 mg of supplemental calcium, found 0.625 mg sufficient to prevent spinal trabecular mineral loss. Piperazine estrone sulfate, 0.625 mg, is considered by most investigators to be equivalent to 0.3 mg conjugated estrogens or to 1 mg of micronized 17β-estradiol because these dosages produce comparable clinical response and similar levels of plasma estradiol.[9] Our study indicates that, at least up to 3 years of treatment, a low dosage of micronized 17β-estradiol, 1.0 mg, is sufficient to prevent bone loss in women whose calcium intake is high (>1000 mg/day).

Proving the synergism between low-dosage estrogen and calcium requires considerable effort. First, the marked interpatient variability in treatment-induced estradiol levels must be overcome by titration of estrogen dosage to achieve a predetermined plasma level. Trial participants must be randomly assigned to low versus high calcium intakes. Institutional approval and recruitment of subjects to be submitted to low calcium intake would be difficult, given the present widespread belief in calcium's salutary effects. Large numbers of women would be needed, and follow-up for several years would be required to find any differences.

Most studies of estrogen's fracture prevention efficacy have examined the impact of estrogen use in the 1960s and 1970s, a time when the average estrogen dosage was equivalent to 0.9 mg of conjugated estrogens.[2] Most women now receive the equivalent of 0.625 mg, a dosage well documented to provide skeletal protection. Although lower dosages appear effective in preventing bone loss, their ability to prevent fractures has yet to be determined. Comparisons made in our study suggest that, in the long term, higher dosages

could provide greater protection against bone loss. Our current and previous[8] studies both found that although many women obtain relief of menopausal symptoms with low-dosage estrogen, some respond better to customary dosages. Furthermore, lower dosages of estrogen have little or no salutary effect on lipoprotein profile[29] and cannot be expected to have the same cardiovascular protective effect as full dosages.

Despite these theoretic and practical drawbacks, low-dosage estrogen may be a useful alternative to higher dosages. Women welcome the reduced amount and frequency of withdrawal bleeding. In spite of considerable evidence that customary dosages of estrogen are not associated with an increased risk of breast cancer,[30] this concern still prompts many women to request the lowest effective dosage. Although the possible synergism between calcium and low-dosage estrogen has not been rigorously proved, a coprescribed high-calcium intake may enhance bone preservation when lower-than-customary doses of estrogen are taken.

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A controlled trial of self–nonstress test versus assisted nonstress test in the evaluation of fetal well-being

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OBJECTIVE: New portable devices have become available for home monitoring of fetal heart rates; these devices have the potential to immediately transmit these tracings to a medical facility. The null hypothesis of this study is the inability of mothers to perform their own nonstress tests (self–nonstress tests) and that such tests are not comparable to those performed by professional medical personnel (assisted nonstress tests).

STUDY DESIGN: The feasibility of maternal self-testing was established in 50 high-risk patients followed by a controlled clinical trial conducted in 60 patients. The latter study represents the first controlled trial in which patients performed self–nonstress tests at their homes and transmitted the tracings via telecommunication to our perinatal unit. In all cases these patients came to our hospital within 60 minutes after the self–nonstress tests to have a perinatal nurse perform a second nonstress test. The pairs of self and assisted fetal heart rate tracings were independently reviewed by two investigators.

RESULTS: The self and assisted tracing pairs were judged satisfactory for interpretation in 100% and 90%, respectively; self and assisted were interpreted by each examiner to be nonreactive in 20% and 14%, respectively. However, both examiners were unable to distinguish between tracings generated by assisted nonstress and self–nonstress tests. Furthermore, cost analysis revealed an estimated twofold savings with self–nonstress testing compared with the assisted nonstress test.

CONCLUSION: Self–nonstress testing is a reliable and accurate method of antepartum fetal heart rate testing. This method of fetal assessment not only introduces a new approach to fetal surveillance with added convenience to patients, but may also significantly reduce medical cost without compromising the results of fetal testing. (Am J Obstet Gynecol 1992;166:489-92.)

Key words: Self–nonstress tests, assisted nonstress tests, antepartum

Antepartum fetal heart rate (FHR) testing is the cornerstone in the assessment of fetal well-being in high-risk pregnancies. As contemporary medicine attempts to maintain optimum care of patients with less inconvenience and to reduce the overall medical cost, various methods of home monitoring are being used. New equipment is now available that is easy to operate and can be used to transmit FHR tracings to medical facilities for review.[1, 2]

Investigators have previously reported successful transmissions of FHR tracings, by medical personnel, from regional hospitals to medical centers for consultation.[3-7] However, the feasibility of maternal self-testing with FHR transmission to a medical facility remained undetermined. In this study, we established the feasibility of self–nonstress testing (NST) in 50 patients and then performed the first controlled clinical trial in 60 patients in which self–NST was compared with assisted NST.

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Material and methods

Feasibility study. High-risk patients undergoing antepartum FHR testing were trained, by a perinatal nurse, to perform self–NST and asked to conduct 30 minutes of FHR tracings. Patients were randomly recruited from the high-risk clinic, which comprises both private and university service patients. Therefore the patient population included both poorly educated and well-educated patients in fairly equal proportions. After an average instruction period of 15 minutes, patients were placed in a private room and asked to conduct self–NST. Tracings were independently and blindly reviewed by two investigators for technical quality and clinical interpretation.

Controlled trial. After the completion of the feasibility study, patients were given home monitors identical to those used in the hospital and were asked to conduct weekly self–NST from their homes. By telecommunication, the FHR tracings were transmitted to the Perinatal Unit (monitoring equipment from Advanced Medical Systems Inc., Hamden, Conn.). In all cases patients returned to our institution within 60 minutes to have a perinatal nurse perform a second non-

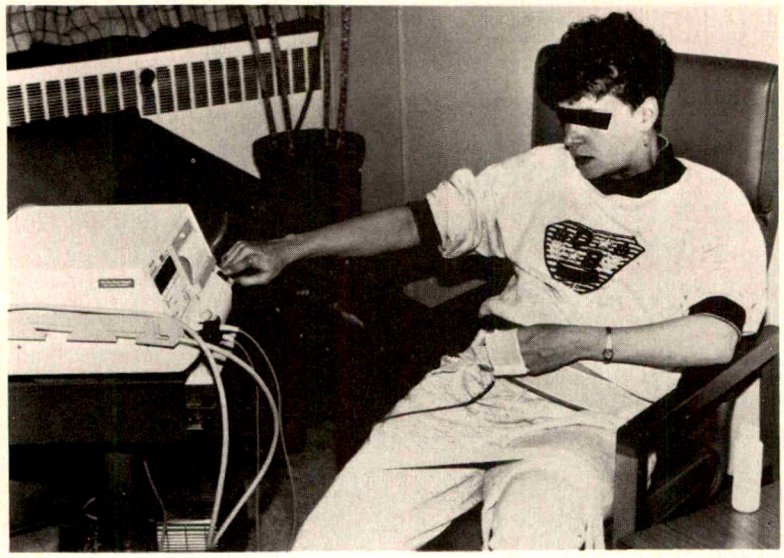


Fig. 1. Patient with twin gestation maintained at bed rest in her home conducted her own NST. She is seen placing belts around the abdomen and trying to find fetal heartbeat.

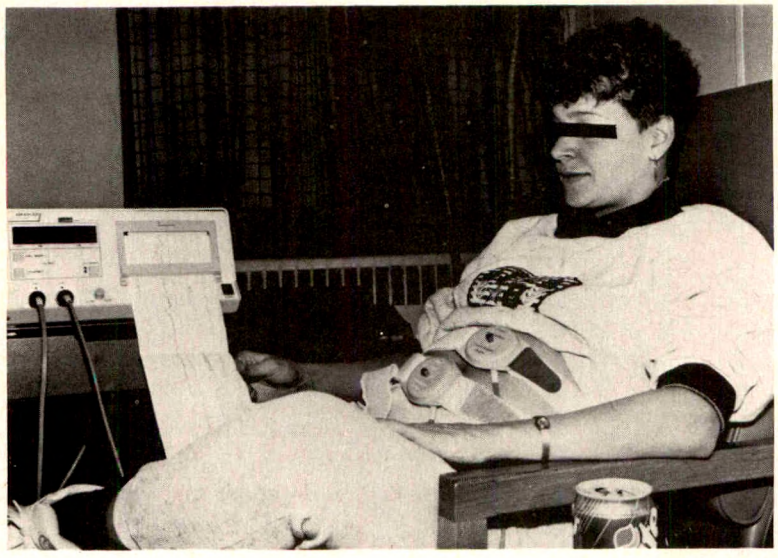


Fig. 2. Patient shown in Fig. 1 has identified FHR. Belts are already strapped around the abdomen, and she is observing dual tracing of both fetuses in progress. A copy of this tracing is being transmitted to the Perinatal Unit of Yale–New Haven Hospital for review.

stress test (assisted NST). The latter test was used for clinical purposes. The physicians and nurses involved in this study did not participate in the patient's high-risk clinical care. The 60 pairs of FHR tracings (both self- and assisted NST) were then independently assessed by two investigators who were blinded to the results of the study.

The fetal monitors used in the study were equipped with an internal memory that was capable of storing up to 80 minutes of heart rate and uterine activity data. The heart rate was digitized at a rate of four samples per second. The stored data were transferred by the telephone line with a 1200-band, half-duplex internal modem. One hour of data was transmitted in approximately 5 minutes. The receiver located in the perinatal unit automatically answered the call from the fetal monitor and recorded the incoming data on a strip chart in a manner similar to the original fetal monitor record (Figs. 1 and 2). The monitor is designed with a timer that allows the ultrasonography to be energized 90 minutes per day only, thus precluding the patient from using the machine all day.

Results

Feasibility study. All 50 patients in the feasibility phase of the study were able to initiate recordings of

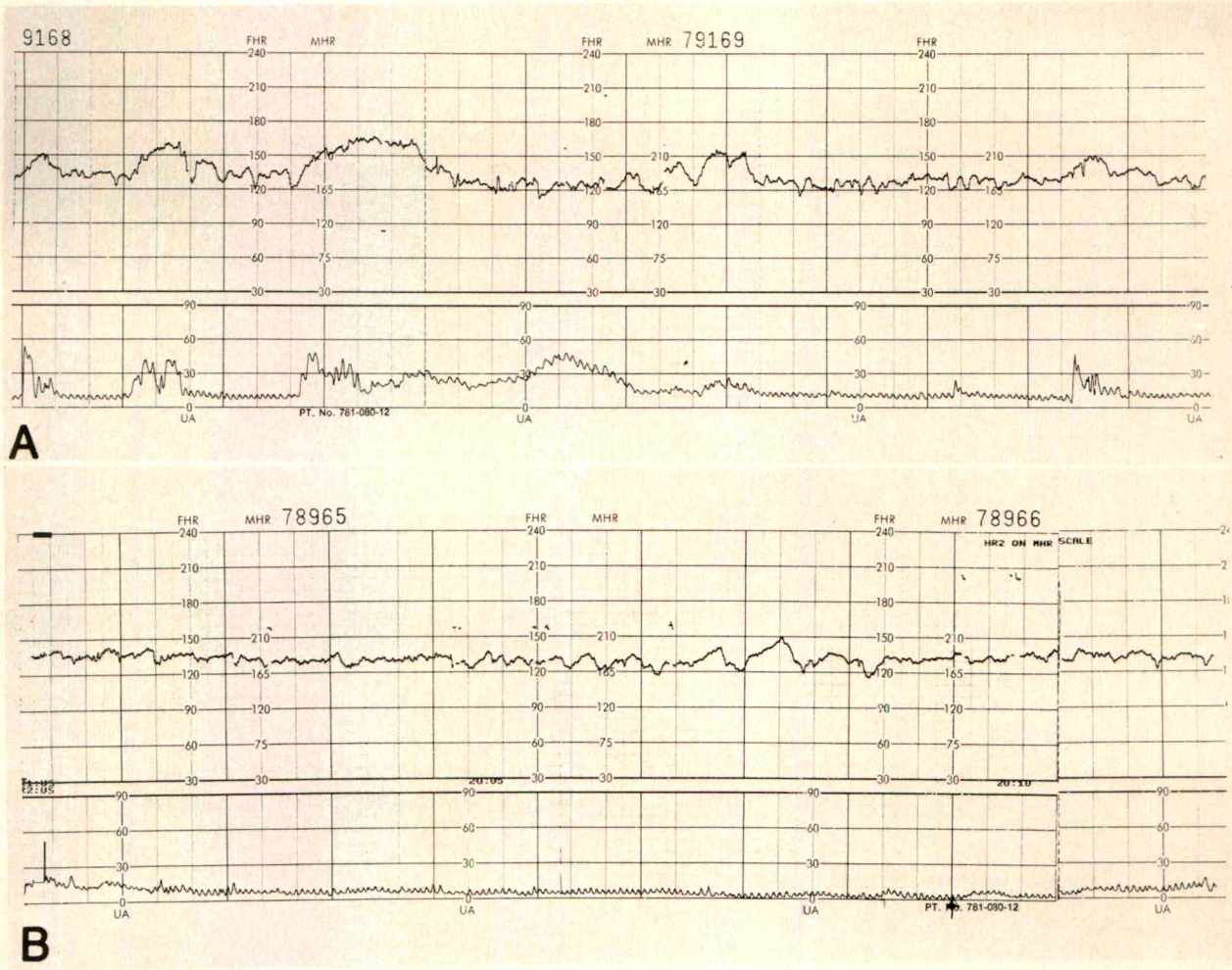


Fig. 3. A, Typical FHR tracing performed by patient (self-NST). **B,** Electronic FHR tracing performed by nursing staff (assisted NST).

the FHR by placing belts around their abdomen and identifying the FHR, then generating a tracing within 5 minutes (Fig. 1). The two investigators, who were blinded to the study, independently assessed the FHR tracings to be satisfactory and adequate for clinical interpretation.

Controlled trial. All patients in this phase of the study were able to successfully perform at-home, weekly self-NST and transmit tracings to our Perinatal Unit (Figs. 1 and 2). A total of 60 pairs of FHR tracings were generated, one performed by the patients at their homes and the other performed subsequently by a perinatal nurse in the hospital. Two investigators independently judged the FHR tracings to be satisfactory for interpretation in 100% and 96%, respectively. Additionally, these tracings were interpreted by each examiner to be nonreactive in 20% and 14%, respectively. However, both examiners were unable to distinguish between tracings generated by assisted NST and self-NST (Fig. 3).

Cost analysis reveals that self-NST would be cost saving by about twofold (Table I).

Comment

In the last decade the widespread use of electronic FHR monitoring in many delivery suites of regional hospitals necessitates the development of systems that may allow direct real-time telephone transmission of FHR signal to receiving units, principally for consultation.

Such systems have improved the quality of perinatal care in regional hospitals.[4, 5] Technologic advances in the last few years have lead to the development of new portable instrumentation that permits home tocodynamometry. This method of home monitoring of uterine activity is currently used in some medical centers as part of preterm delivery prevention programs. Recently, a new technique of transmitting FHR tracings from patients located extramurally to medical facilities has been developed.[2-8] In 1979, Boehm and Haire[7]

Table I. Cost analysis of miniaturized fetal data transmission unit versus regular fetal monitor

At-home program	
Cost of miniaturized date transmission unit	$2000.00
Assumptions:	
Usable life span of 5 yr	
20% Idle time between patients	
1 Test per week (42 per year or 208/5 yr)	
Repairs and transducer replacement costs	+144.00
Cost of NST per unit ($2144/208 for 5 yr)	10.30
Professional fee changed by hospital for reading test	
Approximately one half amount currently charged for outpatient NST because no overhead and technical fee are involved	+59.00
Total cost of NST	$69.00
In-hospital program	
Cost of regular fetal monitor	$4000.00
Assumptions	
Usable life span of 5 yr	
3 per day × 180 days/yr (540 per year or 2700/5 yr)	
Repair and transducer replacement costs.	+502.00
Cost of NST per unit ($4502/2700 for 5 yr)	1.67
Professional fee charged by hospital (includes overhead and technical and professional fees)	+118.00 $120.00
Total cost of NST	$121.67
Additional costs not computed may include:	
Nurse at least 0.5 full-time equivalent for in-hospital program versus nurse at least 0.25 full-time equivalent for at-home program	
Room rental cost or 15 × 15 ft room at $12/sq ft ($2700/yr) (excluding heating, cooling, linen, etc.)	
Cost to the patient	
Lost time from work	
Cost of parking and gas	

showed that using Xerox 400 telecopiers to transmit fetal monitoring data to tertiary centers resulted in an improved ability of the medical staff to make accurate and reliable diagnoses. The Xerox method, however, had major disadvantages, including a limit of 8-minute transmission time and a lack of clarity.

Recently, Vintzileos et al.[3] reported the use of a system that permitted direct transmission by medical personnel of antepartum or intrapartum FHR tracings by a telephone line. By using this option, the authors concluded that it allowed the obstetric staff of smaller community hospitals to obtain an immediate consultation and thus improve the quality of perinatal care. In light of the established use of transmission of FHR tracings and the known ability of mothers to conduct a variety of medical self-tests, including uterine activity monitoring, the possibility of patients performing self-NST and transmitting these tracings to a medical facility seemed attractive. For this reason the current study was initiated.

The results of this study demonstrate not only that it was feasible for mothers to perform antepartum FHR testing on their own fetuses but also that the tracings generated were satisfactory for clinical interpretation. Tracings obtained by either patients or medical personnel were indistinguishable for their accuracy and interpretability. In addition, cost analysis reveals a twofold reduction in cost when this form of fetal testing is used. Previous studies reported on FHR monitoring by telephone have clearly shown that the method functions effectively[2, 6, 8]; however, the current study is the first controlled trial in which self-NST was compared with assisted NST and showed that they are comparable.

In conclusion, this new method of antepartum FHR testing, if used within a structured clinical context, will not only provide accurate and reliable information about fetal well-being but offer patients convenience and the potential for reduced medical costs without compromising the results of fetal testing.

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Angiographic embolization in the management of hemorrhagic complications of pregnancy

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Obstetric hemorrhage continues to be a major cause of maternal mortality and morbidity. Recent developments in percutaneous angiographic embolization techniques have afforded the ability to control persistent bleeding from pelvic vessels while avoiding the morbidity of surgical exploration. We report the use of angiographic embolization in 10 cases of pregnancy-related hemorrhage, including persistent postcesarean bleeding (three cases), vaginal wall hematomas (four cases), cervical ectopic pregnancies (two cases), and postpartum bleeding as a secondary complication of uterine myomas (one case). The embolization procedures were successful in all cases. Nine of 10 patients experienced postprocedural fever with eight cases resolving with antibiotic therapy alone and one patient requiring vaginal drainage of the hematoma-abscess. The mean length of time for the procedure was 167 minutes (range 70 to 270). The average length of hospitalization was 8 days (range 2 to 13). These data indicate that angiographic embolization is effective in treating hemorrhagic complications of pregnancy in hemodynamically stable patients and is preferable to surgery in selected cases. (Am J Obstet Gynecol 1992;166:493-7.)

Key words: Angiographic embolization, hemorrhage, pregnancy

Obstetric hemorrhage remains one of the three main causes of maternal death in spite of modern blood banks, anesthesia, and pharmacologic agents to treat such complications. Effective management requires prompt identification of the cause and usually responds to one of several targeted conservative modalities, such as oxytoxics, blood component therapy, and careful surgical technique. However, persistent bleeding may occur after the evacuation of a vaginal hematoma or cesarean hysterectomy and may also result from postpartum uterine atony associated with myomas. Cervical ectopic pregnancies, although rare, represent a particularly challenging problem of hemostasis, particularly if unrecognized before curettage.

Hypogastric artery ligation has traditionally been the surgical approach when exploration and arterial ligation prove unsuccessful. With the development of radiographic arterial embolization techniques, an alternative to exploratory surgery or hypogastric artery ligation has become available. The use of angiographic embolization to control hemorrhage has been well described in patients with gynecologic cancer and postpartum vaginal wall hematomas.[1-6] However, in spite of the availability of this technique in many hospitals throughout the United States, it is not widely used on most obstetric services. In this study we report our experience with angiographic embolization in various clinical hemorrhagic settings to emphasize its use as compared with hypogastric artery ligation.

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Material and methods

Between July 1984 and December 1990, 10 patients at the University of California at San Diego Medical Center and Mercy Hospital and Medical Center required angiographic embolization to control obstetric hemorrhage. All hospital records were reviewed and detailed to collect pertinent clinical data pertaining to the specific leading complication, clinical status, blood replacement requirements, length of embolization procedure, and complications. Two of these cases (7 and 8, Table I) have been reported previously.[1]

Angiographic consultation and intensive care facilities are available on a 24-hour basis at both of these hospitals. Several patients were referred to our institutions from outside hospitals where these embolization techniques were not available. All patients with hemorrhagic complications unresponsive to conservative management were stabilized and taken to the radiology suite for the angiogram and embolization. By the percutaneous approach with the patient under local anesthesia, an aortogram was performed for identification of the pelvic arterial anatomy and bleeding sites. A variety of catheter shapes can then be used to enable selective catheterization of the bleeding artery, but one

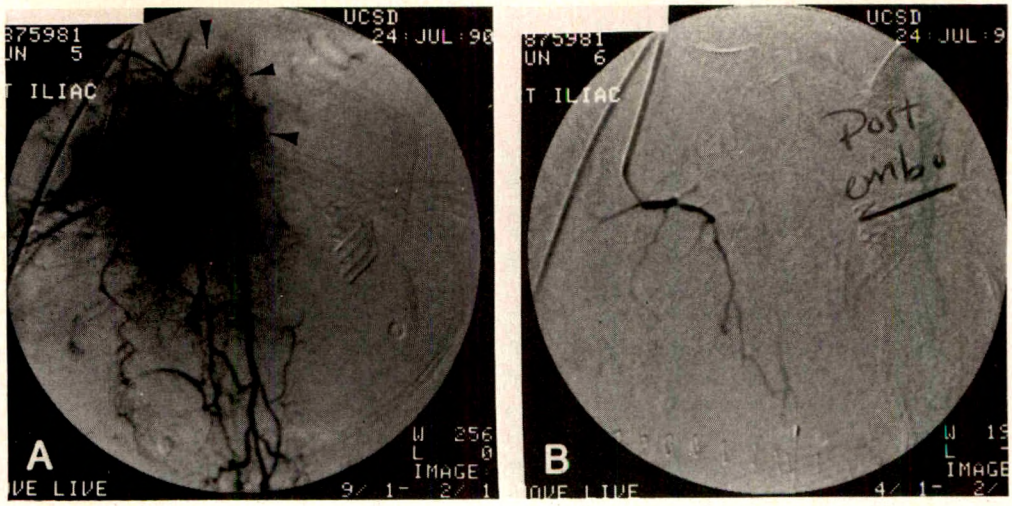


Fig. 1. A, Preembolization arteriogram demonstrates free bleeding in area of vaginal cuff after cesarean hysterectomy for uterine atony (case 6). This free bleeding is described as "blush" *(arrows)* and demonstrates significant bleeding. **B,** Postembolization film demonstrates complete blockage of hypogastric artery on right after the Gelfoam injection.

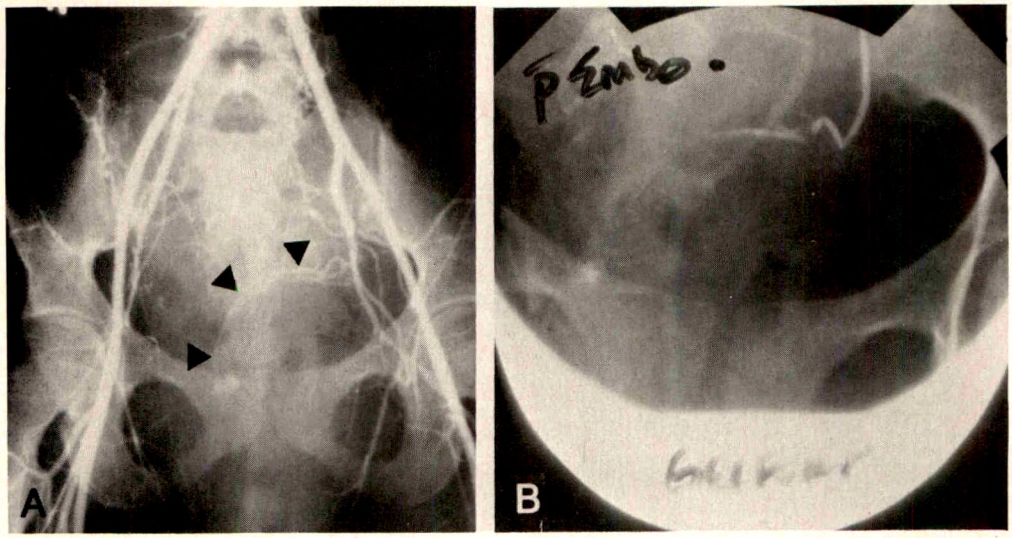


Fig. 2. A, Preembolization angiogram demonstrates outline of vaginal hematoma *(arrows)* from case 10. **B,** Postembolization film demonstrates complete blockage of left vaginal branch and pudendal branch of hypogastric artery.

of us (J.J.B.) strongly recommends the long-reversed-curved catheter of Cordis (Cordis Inc., Miami). Subselective embolization of the bleeding vessel was then performed, usually with small pieces (2 × 2 × 2 mm) of Gelfoam (Upjohn, Co., Kalamazoo), through the catheter. Gelfoam is a gelatin sponge that is slowly absorbed and ultimately enables recanalization of the embolized vessel. Hemostasis was documented by fluoroscopic monitoring. If bleeding continued, further embolization was performed until the bleeding stopped. The catheter was then switched to the opposite hypogastric artery and the same procedure as above was performed to provide hemostasis if needed. A final aortogram was performed to document overall hemostasis and to exclude the possibility of continued bleeding from collateral sources. If necessary, collaterals themselves were then embolized.

Results

A summary of the 10 patients requiring embolization is shown in Table I. The average length of the angiographic procedure was 167 minutes (range 70 to 270).

Table I. Ten cases of angiographic embolization

Case No.	*Gestational age (wk)*	*Diagnosis*	*Length of procedure (min)*	*Blood*	*Complications*	*Hematocrit (%)*	*Procedure*	*Length of stay (days)*
1	7	Cervical ectopic	155	9 units PRBC	Fever, disseminated intravascular coagulation	28.3	Bilateral hypogastric artery embolization	5
2	40	Uterine atony	165	6 units PRBC	Fever, disseminated intravascular coagulation	18.1	Bilateral hypogastric artery embolization	8
3	40	Uterine atony	150	Refused	Fibroids, pregnancy-induced hypertension	22.5	Bilateral hypogastric artery embolization	3
4	12	Cervical ectopic	70	2 units PRBC	Fever, disseminated intravascular coagulation	29.3	Bilateral hypogastric artery embolization	2
5	38	Vaginal hematoma	165	7 units PRBC	Fever	19.4	Bilateral hypogastric artery embolization	6
6	35	Vaginal bleeder	240	18 units PRBC	Cesarean hysterectomy for atony	21	Bilateral hypogastric artery embolization	12
		Bleeding	210		Persistent vaginal bleeding		Right ovarian	
7	40	Vaginal hematoma	120	Refused	Fever, pelvic cellulitis	13	Right hypogastric artery embolization	13
8	40	Vaginal hematoma	270	33 units PRBC	Fever, liver dysfunction shock, disseminated intravascular coagulation	18	Bilateral hypogastric artery embolization	11
		Bleeding					Right ovarian	
9	39	Uterine atony	??	Refused	Fever, chronic pelvic pain	15	Bilateral hypogastric artery embolization	6
10	39	Vaginal hematoma	90	4 units PRBC	Fever, drainage of hematoma	21.6	Left hypogastric artery embolization	11

PRBC, Packed red blood cells. Cases 6 and 8 had two angiographic embolizations performed on separate days.

The time of the procedure is recorded from that time the patient was sent to the angiographic unit until her return to the obstetric or intensive care unit. However, the time required to control bleeding usually is much shorter (but less well documented) and was only 25 minutes from start of procedure to control of bleeding in case 10. The primary complication included postprocedural fever in nine of 10 cases, eight of nine responding to antibiotics alone, whereas in case 7 vaginal drainage of a paravaginal abscess cavity was required 8 days after radiographic embolization. Seven of 10 patients required blood transfusions before or after the procedure, and the other three patients refused blood products (cases 3, 7, and 9).

In case 6 the patient had undergone a cesarean hysterectomy because of uterine atony associated with a triplet delivery. The estimated blood loss during and after surgery was 3000 ml, and the patient was transfused with 18 units of packed red blood cells and multiple units of fresh-frozen plasma. The patient continued to experience vaginal bleeding after surgery and was taken to the radiology suite where angiography revealed bleeding in the area of the vaginal cuff, as demonstrated by the dye "blush" outlined by the arrows in Fig. 1, *A*. Bilateral hypogastric artery embolization was accomplished (Fig. 1, *B*), and adequate hemostasis was felt to have been obtained. However, the patient continued to have bleeding from drains and underwent

a second angiogram the following day to determine whether the ovarian arteries could be a possible source for the continued bleeding. Embolization of the right ovarian artery was successful in obtaining hemostasis.

In case 3 the patient underwent a normal vaginal delivery and was found to have continued bleeding in spite of a firm, well contracted uterus and postpartum curettage. The patient was known to have myomas. She underwent angiograms which revealed uterine bleeding. Embolization of the anterior division of both hypogastric arteries was successful in stopping the hemorrhage.

The patient described in case 10 was transferred after a traumatic forceps delivery, which resulted in an expanding left vaginal hematoma. In Fig. 2, *A*, the hematoma is outlined by the arrows. Embolization was directed into the left hypogastric artery, and follow-up x-ray films (Fig. 2, *B*) reveal that the bleeding has stopped. Two days later the patient was taken to the operating room, where the hematoma was uneventfully drained. The patient did quite well and went home on the eleventh hospital day.

Case 8 involved a patient who presented with signs of severe pregnancy-induced hypertension and possible fatty liver of pregnancy, including liver failure and disseminated intravascular coagulation. After a forceps delivery, an expanding hematoma that was noted on the left vaginal wall did not respond to conservative exploration and packing. The patient was subsequently transferred to our institution where additional conservative treatment, including two additional attempts at vaginal packing, was unsuccessful. The patient then underwent embolization of the left and right hypogastric arteries, which initially controlled the bleeding. However, because of recurrent hemorrhage into the paravaginal space and the question of a retroperitoneal hematoma, she had a second procedure that embolized the remaining vessels of the left hypogastric artery and right ovarian artery.

Comment

These cases illustrate the role of angiographic embolization as an alternative to surgery for the treatment of obstetric hemorrhage from a variety of causes. Angiographic embolization prevented the need for a general anesthetic, allowed the preservation of reproductive capability, and prevented reexploration in those patients who had undergone surgery. The complications related to the procedure were minimal and included postprocedural fever, which responded to antibiotic therapy, and transient buttock pain, which resolved over time. Angiographic embolization has been reported effective at stopping arterial hemorrhage in >90% of cases of gynecologic hemorrhage because of the ability to place the embolic material within the hypogastric artery.[7, 8] In our cases, success was achieved after the first attempt at embolization in eight of 10 cases, whereas an additional procedure to produce hemostasis was necessary in two cases.

Hypogastric artery ligation is often recommended as the primary conservative surgical procedure to control hemorrhage in similar complications. The primary goals of hypogastric artery ligation are to preserve reproductive function and to offer less morbidity than more definitive surgery. Clark et al.,[9] however, found that hypogastric artery ligation was successful in only eight of 19 (42%) patients with obstetric hemorrhage. Bleeding failed to stop in the remaining 11 patients, and hysterectomy was required.[9] Furthermore, those patients in that series who underwent hysterectomy as the primary procedure had substantially less morbidity than those who underwent hypogastric artery ligation and hysterectomy. Hypogastric artery ligation decreases arterial pulse pressure by 85% distal to the ligation but decreases blood flow in these distal vessels by only 48%.[10] The extensive collateral circulation in the pelvis maintains a significant blood flow in spite of the ligation. This may well account for the low success rate of hypogastric ligation in providing adequate hemostasis. Embolization, however, enables specific obstruction of the bleeding vessel and produces obstruction beyond entry of large collaterals. Angiographic embolization must be considered before hypogastric artery ligation, because once the artery is ligated, embolization cannot be performed.

Obstetric hemorrhage can often be extremely rapid, with exsanguination and death occurring within minutes. In these emergency cases the patient must be stabilized and the bleeding stopped by the most rapid and complete method possible. If conservative methods such as applied pressure or packing are sufficient to provide temporary hemostasis, the patient may be stabilized with intravenous fluids, blood replacement, and clotting factors, if indicated. Resuscitation status is monitored by measuring the arterial blood gas and base excess, hematocrit, and urine output. If a coagulopathy is present, it should be corrected before or during embolization. The angiographic team should be consulted early in the course of a hemorrhagic complication to allow time for the team to arrive and prepare to treat the patient. Complications associated with angiographic embolization include embolization of an inappropriate vessel or ischemia distal to the embolization. These complications are rare because of the rich collateral blood supply in the pelvis. In our cases fever occurred in nine of 10 cases. All but one responded to antibiotic therapy alone, whereas one case required vaginal drainage of an abscess 8 days after the embolization.

Angiographic embolization should also be consid-

ered in cervical ectopic pregnancy or if bleeding ensues when this complication has gone unrecognized. Under optimal circumstances, consideration should be given first to the use of new treatment modalities, such as methotrexate, to treat such ectopic gestation.[11, 12]

Our experience demonstrates that angiographic embolization is a safe, effective, and relatively rapid procedure that should be considered early in the course of obstetric hemorrhage that is unresponsive to conservative management. If the patient is hemodynamically stable, the procedure should be performed before hypogastric artery ligation or hysterectomy.

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Breech extraction of low-birth-weight second twins: Can cesarean section be justified?

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The outcomes of 54 breech-extracted second twins weighing between 750 and 2000 gm were compared with the outcomes of their siblings and of 43 sets of twins delivered by cesarean section for malpresentation. Vaginally delivered first twins had fewer days of mechanical ventilation and oxygen therapy than their breech-extracted siblings ($p = 0.004$). There were no significant differences in any measures of neonatal outcome when breech-extracted twins were compared with second twins delivered by cesarean section. We conclude that routine cesarean section is not justified for nonvertex second twins expected to weigh <2000 gm. (Am J Obstet Gynecol 1992;166:497-502.)

Key words: Breech extraction, cesarean section, twins, low birth weight

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The optimal route of delivery for low-birth-weight vertex and nonvertex twin pairs is a matter of obstetric controversy. The incidence of such pairings is between 38% and 51% and is inversely related to gestational age.[1-3] Although there is evidence to support the safety of vaginal delivery with breech extraction of the second

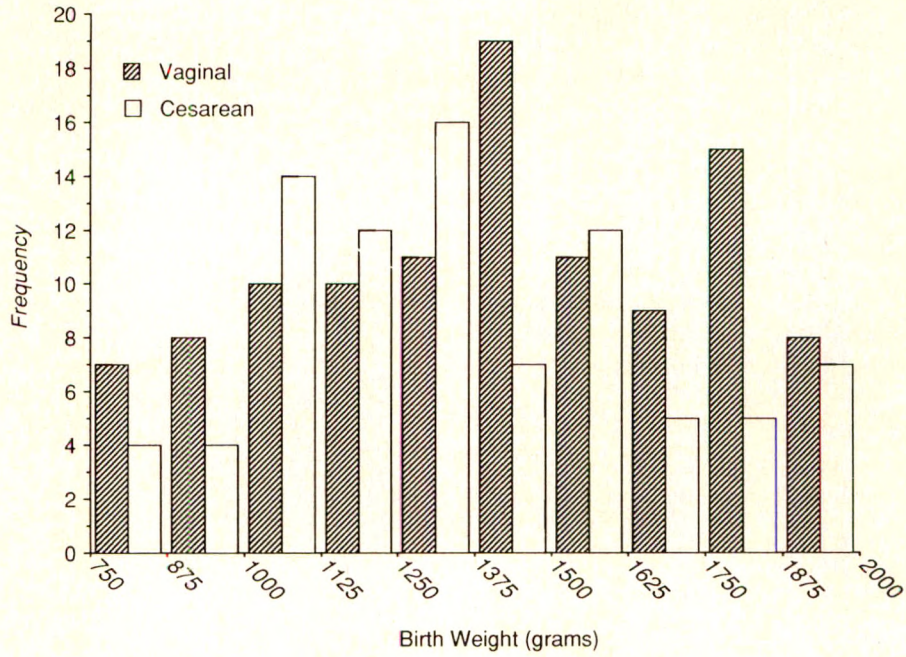


Fig. 1. Distribution of birth weights by route of delivery.

Table I. Delivery mode distribution

Delivery mode (Twin A/Twin B)	*Number*
Vaginal/breech extraction	54 (29%)*
Cesarean/cesarean for presentation	43 (21%)*
Vaginal/vaginal vertex	58 (29%)
Cesarean/cesarean for other indications	38 (19%)
Vaginal/cesarean	8 (4%)

*Study subjects.

twin for those with birth weights >1500 gm,[4-6] there are few reports with specific data regarding the management of lower birth weight twins.

Since 1979 many of the obstetricians at University of Washington Medical Center have chosen to manage the delivery vertex and nonvertex twin pairs by vaginal trial with breech extraction of the second twin, regardless of estimated birth weight.

The purpose of this retrospective study was to evaluate morbidity and mortality in low-birth-weight breech extracted second twins and to determine whether routine cesarean section is justified in this group.

Material and methods

We hypothesized that breech extraction of low-birth-weight second twins is not associated with excess morbidity or mortality sufficient to justify routine cesarean section for nonvertex presentation of a second twin. To test this hypothesis we identified a population of second twins delivered by breech extraction and compared their outcomes with the outcomes of their twin siblings and the outcomes of a comparable population of second twins delivered by cesarean section for the indication of malpresentation. Since both infants within a twin pair are usually managed by the same route of delivery, we also compared the outcomes of all twins (As and Bs) delivered vaginally with the outcome of all twins (As and Bs) delivered by cesarean section.

Charts of women delivering at the University of Washington Medical Center between July 1979 and March 1990 were reviewed to identify twin pairs with both infants born alive, without serious congenital anomaly, and weighing between 750 and 2000 gm. The study groups thus consisted of vaginally delivered twin pairs in which breech extraction was performed for the second twin and twin pairs delivered by cesarean section for malpresentation of the first or second twin.

Data were collected by chart review and from the neonatal data base. Measures of neonatal outcome were survival rates; the incidence of intracranial hemorrhage grades III and IV[7]; necrotizing enterocolitis; infant respiratory distress syndrome; and the duration of hospitalization, mechanical ventilation, and oxygen therapy.

In the cases in which twin A was delivered vaginally and twin B underwent total breech extraction, deliveries were performed under double set-up conditions by a resident with supervision by a faculty member. Pediatricians were present for neonatal resuscitation.

Table II. Neonatal outcome by delivery mode (mean ± SD)

	Vaginal A and B	*Cesarean A and B*	*p*
N	108	86	
Birth weight (gm)	1415 ± 334	1394 ± 328	NS
Gestational age (weeks)	30.2 ± 2.6	30.1 ± 2.5	NS
Survival	96 (89%)	79 (92%)	NS
Infant respiratory distress syndrome	68 (63%)	53 (62%)	NS
Necrotizing enterocolitis	7 (6.5%)	4 (5.8%)	NS
Severe intracranial hemorrhage	10 (9.3%)	10 (12%)	NS

NS, Not significant ($p < 0.05$).

The mother had anesthesia, usually epidural, for pain control and to facilitate manipulation. After spontaneous vaginal delivery of twin A, twin B's presentation was determined by real-time ultrasonography, and total breech extraction was initiated immediately, according to standard techniques.[8]

The data were analyzed by the Student *t*, χ^2, Fisher's exact, Wilcoxon, and Mann-Whitney tests.

Results

Over the 10-year period, 201 twin pairs met initial study criteria. Delivery modes are outlined in Table I. In 54 pairs, vaginal delivery of the first twin was followed by breech extraction of the second twin. In 43 pairs, cesarean section was performed for malpresentation of one or both twins. These two sets of twins were the subjects of the study.

We excluded from consideration the 58 twin pairs in which both twins were vertex and had spontaneous vaginal deliveries, and the 38 pairs delivered by cesarean section for maternal or fetal medical indications such as distress, hypertension, bleeding, or cord prolapse. We also excluded the eight pairs in which twin A was delivered vaginally and twin B required emergency cesarean section. In four cases, the second twin was vertex and was delivered by cesarean section due to abruptio placentae (two) or failure to descend (two). In three cases, external version of the second twin was unsuccessfully attempted, and breech extraction was not considered as a management option. In one case, breech extraction was not undertaken because the cervix was found to be only 8 cm dilated after delivery of the first twin.

Four groups of infants were compared: breech-extracted second twins (vaginal Bs), their siblings (vaginal As), and the twins delivered by cesarean section for presentation (cesarean As and cesarean Bs). Breech-extracted infants were compared both with their siblings and with second twins delivered by cesarean section. The vaginally delivered twins were also compared as a group with those delivered by cesarean section.

There were no cases of birth trauma (e.g., brachial nerve palsy), fracture, or head entrapment. None of the infants were growth-retarded. Birth weights were equally distributed among the study groups (Fig. 1). There were no differences in survival, mean birth weight, or gestational age or in the incidence of infant respiratory distress syndrome, necrotizing enterocolitis, or severe intracranial hemorrhage among infants grouped by route of delivery only (Table II). Table III displays similar data with infants grouped by route of delivery and birth order; there were no significant differences among the groups in any of these variables, except that second twins delivered by cesarean section (cesarean Bs) had a greater incidence of infant respiratory distress syndrome than their siblings (cesarean As) ($p = 0.05$).

Duration of mechanical ventilation, oxygen therapy, and hospitalization were nonparametrically distributed and are displayed graphically in Figs. 2 and 3. In these figures, the percentile rank of each infant is plotted against the outcome variable. When infants were grouped by route of delivery only (Fig. 2), there were no differences in morbidity. When infants were grouped by route of delivery and birth order (Fig. 3), paired tests were used when siblings were compared. Breech-extracted second twins (vaginal Bs) were found to have a significantly greater duration of mechanical ventilation and oxygen therapy than vaginally delivered first twins (vaginal As) ($p = 0.004$). When breech-extracted second twins were compared with second twins delivered by cesarean section, no significant differences were found.

Results were unchanged when data were analyzed to exclude infants who weighed >1500 gm. The incidence of infant respiratory distress syndrome in vaginal Bs was 81.3% compared with 80% for cesarean Bs ($p = 0.80$). The incidence of grade III or IV intracranial hemorrhage was 12.5% compared with 20% ($p = 0.60$). The survival rates were 82% compared with 90% ($p = 0.53$). The results also were unchanged when nonsurvivors were excluded.

Comment

Breech extraction for second twins with birth weights >1500 gm has not been associated with increased neo-

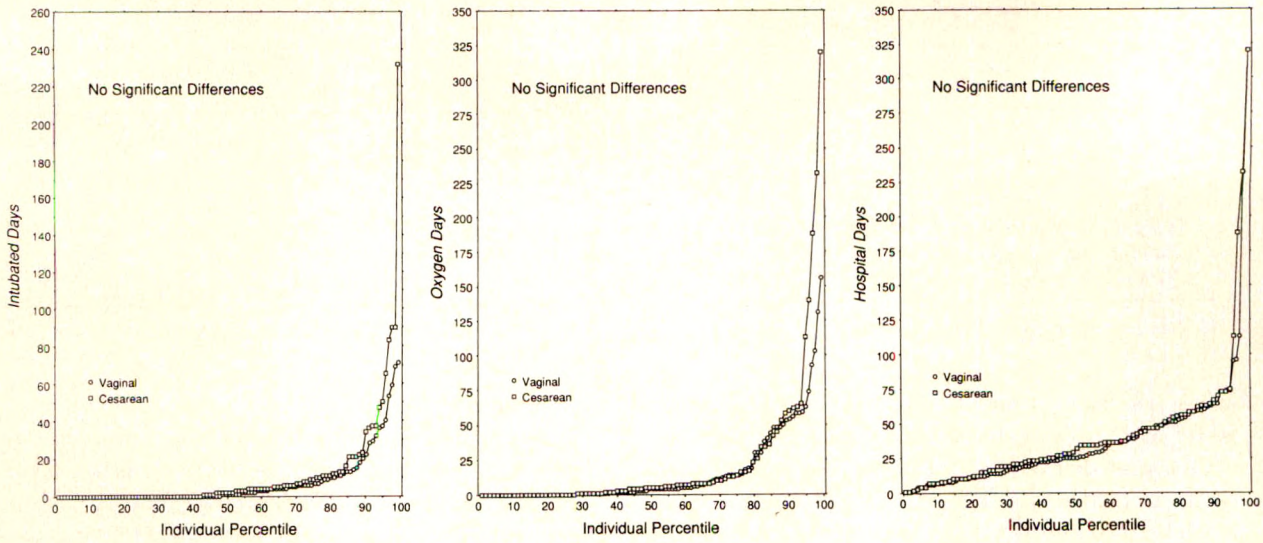


Fig. 2. Duration of mechanical ventilation, oxygen therapy, and hospitalization by route of delivery. Duration of therapy for each infant is plotted against infant's percentile rank within its group. Percentile ranks are used to adjust for unequal sizes of groups.

Table III. Neonatal outcome by birth order and delivery mode (mean ± SD)

	Vaginal A	P_1	*Vaginal B**	P_2	*Cesarean B*	P_3	*Cesarean A*
N	54		54		43		43
Birth weight (gm)	1409 ± 325	NS	1422 ± 345	NS	1386 ± 330	NS	1401 ± 330
Survived	49 (91%)	NS	47 (87%)	NS	41 (96%)	NS	38 (88%)
Infant respiratory distress syndrome	30 (56%)	NS	38 (71%)	NS	31 (72%)	0.05	22 (53%)
Necrotizing enterocolitis	5 (9.3%)	NS	2 (3.7%)	NS	5 (12%)	NS	0 (0%)
Severe intracranial hemorrhage	5 (9.3%)	NS	5 (9.3%)	NS	6 (14%)	NS	4 (9.3%)

P_1, Vaginal A vs vaginal B (breech-extracted); P_2, vaginal B (breech-extracted) vs cesarean B; P_3, cesarean B vs cesarean A; *NS*, not significant ($p < 0.05$).

*Breech extracted.

natal morbidity or mortality.[4-6] Cesarean section has not been recommended for infants of this birth weight category.

Breech extraction of second twins weighing <1500 gm has not been well studied. Chervenak et al.[6] reported 76 breech-extracted second twins of whom 16 weighed <1500 gm; they found that delivery mode was not associated with any difference in outcome. Nevertheless, they recommended routine cesarean section for breech infants with birth weights <1500 gm. Barrett et al.[9] found that breech-extracted second twins weighing <1500 gm had lower Apgar scores and increased neonatal morbidity when compared as a group with their first born siblings. This difference was not found among twins delivered by cesarean section. Although comparisons were not made between second twins delivered vaginally and those delivered by cesarean section, the authors recommended routine cesarean section be undertaken for nonvertex twins expected to weigh <1500 gm.

We found that vertex first twins delivered vaginally (vaginal As), required fewer days of mechanical ventilation and oxygen therapy than second twins who were delivered by breech extraction (vaginal Bs). This is consistent with Barrett et al.'s findings that first twins have fewer complications than their siblings.[9] However, we found no differences between second twins delivered by breech extraction (vaginal Bs) and second twins delivered by cesarean section (cesarean Bs) (Table III and Fig. 3). The outcomes of second twins delivered by breech extraction were comparable to the outcomes of first and second twins delivered by cesarean section. The differences observed among the four groups seem to be attributable to an improved outcome associated with labor and vaginal delivery for vertex first twins.

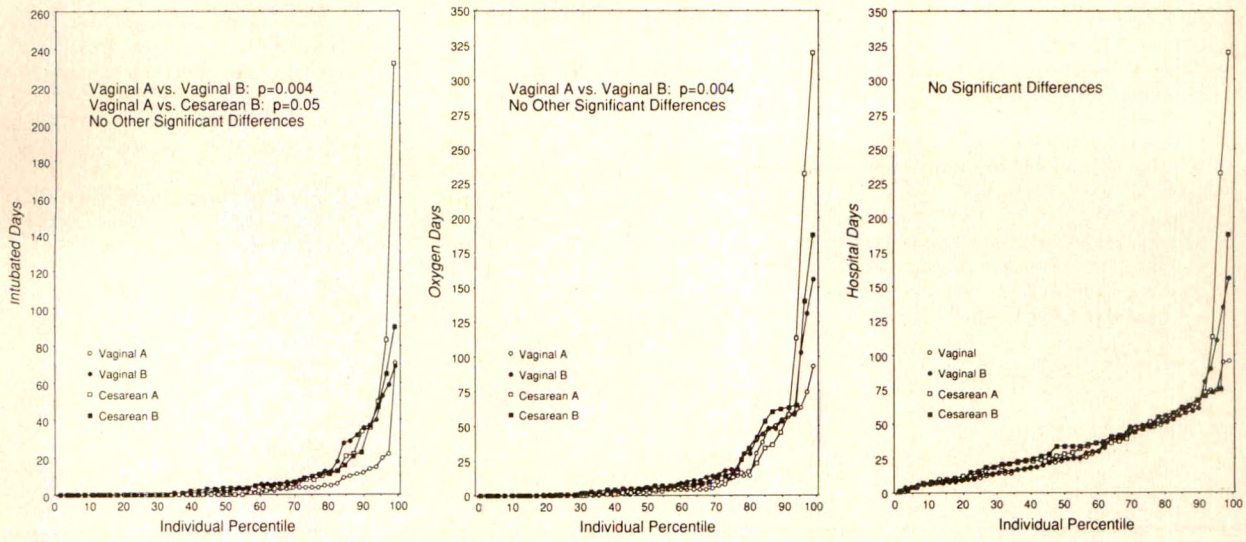


Fig. 3. Duration of mechanical ventilation, oxygen therapy, and hospitalization by route of delivery and birth order. Duration of therapy for each infant is plotted against infant's percentile rank within its group. Percentile ranks are used to adjust for unequal sizes of groups.

Figs. 2 and 3 permit a careful inspection of the performance of the individuals in each group and a visual comparison of the groups as a whole. The data are presented in this fashion because they are not parametrically distributed; means and standard deviations would not be appropriate. When significant differences do exist, they are due to differences in the most severely affected fourth of the subjects. Therefore, in spite of high levels of significance between some groups, the medians of these are nearly identical. Finally, the nonparametric nature of the data precludes an estimate of power. When comparisons are not significant, the reader can see that the outcome curves are superimposed and that the probability is small that clinically significant differences could be found with a larger sample size.

In Fig. 2, which compares infants by route of delivery alone (vaginal As and Bs compared with cesarean As and Bs), the curves are nearly indistinguishable. When twins were compared by birth order and route of delivery, first twins delivered vaginally (vaginal As) were found to require significantly fewer days of mechanical ventilation than their breech-extracted siblings (vaginal Bs). The curves for the other three groups of infants are nearly indistinguishable. When duration of oxygen therapy is considered, first twins delivered vaginally required less therapy than breech-extracted infants. However, inspection of the curves suggests that the differences which are statistically significant may be too small to be clinically significant.

The weaknesses of a retrospective study design are well known, but a randomized, controlled trial of the effect of breech extraction on the neonatal outcome of low-birth-weight second twins is impractical. However, we compared a relatively large number of breech extracted second twins with their siblings and with second twins delivered by cesarean section. The nonsignificant differences we observed are trends toward decreased morbidity for the vaginally delivered twins. This suggests that, although an even larger study group might be needed to avoid a type II error, we have probably not failed to detect any tendency toward an increase in morbidity attributable to breech extraction. By excluding from consideration those twin pairs delivered by cesarean section for medical indications other than presentation, we have avoided the possibility of introducing a bias toward worse neonatal outcomes in the cesarean section group.

Cesarean section is an intervention that should not be undertaken without justification on the basis of an objective risk-benefit analysis. We were unable to demonstrate that cesarean section improved neonatal outcome when breech-extracted second twins were compared with second twins delivered by cesarean section. In addition, vaginal delivery was associated with an improved outcome for vertex first twins. We therefore conclude that routine cesarean section is not justified for nonvertex second twins expected to weigh <2000 gm.

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Cervical stenosis in pregnancy: A complication of cryotherapy in diethylstilbestrol-exposed women

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Cryotherapy causes a high incidence of cervical stenosis in diethylstilbestrol-exposed women because of an abnormal healing response. Consequently, the cervix may not dilate during labor, necessitating cesarean section. To prevent this, therapy should be localized to the abnormal tissue on the cervix instead of the entire transformation zone whenever possible. (Am J Obstet Gynecol 1992;166:502-3.)

Key words: Cryotherapy, diethylstilbestrol, cervical stenosis, cervical dystocia

Two cases are reported in which cervical stenosis was diagnosed during labor in diethylstilbestrol (DES)-exposed women who previously had cryotherapy for cervical intraepithelial neoplasia.

Case reports

Case 1. A 25-year-old DES-exposed woman had cryotherapy for grade 1 cervical intraepithelial neoplasia 18 months previously. On her initial examination during pregnancy, the cervix appeared short and broad; the external os was not well defined.

She was admitted at term, 1 hour after spontaneous rupture of the membranes, with painful uterine contractions every 2 minutes. The cervix was difficult to outline. It felt like there was a small dimple in the middle of the vaginal apex, and the lower uterine segment was pushed downward ahead of the vertex, which was engaged. The cranial sutures could be palpated through the thin lower uterus.

Speculum examination demonstrated amniotic fluid coming from the top of the vagina but no cervical os was visualized. The anterior cervix appeared edematous, as if fluid had been injected underneath the mucosa.

Painful uterine contractions continued, consistent with active labor. Cervical stenosis was suspected, so a low transverse cesarean section was done with the patient under epidural anesthesia. The 7-pound 10-ounce male infant had marked molding. The lower uterine segment was very thin. From above, the internal os felt just like a dimple. A small hemostat could be passed through it into the vagina. During this, it was evident that the cervix had effaced completely.

At examination 6 weeks post partum the cervix was still difficult to outline distinctly, although it was possible to obtain a cervical cytologic sample, including endocervical brush sampling. Severe dysmenorrhea, which was present before cryotherapy, was relieved by oral contraceptives.

Case 2. A 31-year-old DES-exposed woman had

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cryotherapy for grade 1 cervical intraepithelial neoplasia. On the initial pregnancy examination 6 years later, the cervix did not appear abnormal.

Labor started at 39 weeks. After 2 hours of regular contractions, the external os was a dimple and could not be entered by digital examination. The uterine contractions became painful and continued every 5 minutes for 9 hours. The external os did not dilate. The vertex was at −2 station, well applied to the cervix. Cervical stenosis was diagnosed, and a low transverse cesarean section was done with the patient under epidural anesthesia. The 8-pound 4-ounce female infant had some molding. The lower uterine segment was very thin. The cervical os could not be probed from above with a finger or a narrow curved clamp.

At examination 6 weeks post partum the cervix did not appear abnormal. Dysmenorrhea, which was present before and after cryotherapy, was minimal after childbirth.

Comment

The effects of in utero DES exposure on the reproductive tract have been well described. They include structural abnormalities of the uterine corpus and cervix that can lead to spontaneous first-trimester and midtrimester abortion and preterm labor.

In managing the labors of the cases reported above, clinically there was no question that there was cervical stenosis and that cesarean section was necessary. I have managed other cases in which cervical stenosis caused dystocia in labor, such as conglutination after abortion attempts with potassium permanganate or from scarring due to previous conization. The cervix effaces as the internal os and canal are drawn upward around the head by the uterine contractions, but the external os remains a dimple and the lower uterine segment balloons downward into the vagina, ahead of the presenting part. In some of these cases the cervix can be dilated with a uterine dressing forceps and then labor can continue normally.

In these two cases it was assumed that the cervical stenosis was related to the DES exposure and cryotherapy. The incidence of cervical stenosis associated with cryotherapy in patients exposed in utero to DES is 75% to 85%.[1] Cryotherapy is usually a low-risk procedure. However, in DES-exposed women there is an abnormal healing response of the cervix to cryosurgery or conization.[2] In these two patients the freeze, which was done with the short-tip exoprobe, was shallow, involving only the external os and not much of the cervical canal, so they had no difficulty conceiving.

The unique aspect of these cases is that they demonstrate the effect that in utero DES exposure plus cryotherapy can have on the ability of the cervix to dilate during labor. When treating cervical intraepithelial neoplasia in DES-exposed patients who desire children, one should consider locally destructive procedures such as laser aimed at treating the demonstrable dysplastic tissue, if possible, rather than the entire transformation zone.[1, 2]

DES-associated problems represent a unique occurrence in medicine in that they will become obsolete. In the meantime we must continue to deal with their consequences. This report adds one more potential obstetric complication.

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Meta-analysis of surgery in advanced ovarian carcinoma: Is maximum cytoreductive surgery an independent determinant of prognosis?

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OBJECTIVE: If maximum cytoreductive surgery benefits the survival of women with advanced ovarian cancer, the median survival time of groups of such women will improve as the proportion of women undergoing maximum cytoreductive surgery is increased.

STUDY DESIGN: Fifty-eight suitable studies that encompass 6962 patients with advanced ovarian cancer were identified. Multiple linear regression was used to analyze the effects on median survival time of the following variables: the proportion of each cohort undergoing maximum cytoreductive surgery, the use of platinum-containing chemotherapy, the dose intensity of chemotherapy, the proportion of each cohort with stage IV disease, and the year of publication of the study.

RESULTS: Maximum cytoreductive surgery was associated with only a small improvement in median survival time, but platinum-containing chemotherapy improved median survival time substantially. Increased dose intensity also conferred a useful survival benefit.

CONCLUSION: Cytoreductive surgery probably has only a small effect on the survival of women with advanced ovarian cancer. The type of chemotherapy used is more important. (Am J Obstet Gynecol 1992;166:504-11.)

Key words: Maximum cytoreductive surgery, advanced ovarian carcinoma, prognosis, chemotherapy

Most ovarian cancer is first seen as advanced intraabdominal disease. Complete surgical extirpation can rarely be achieved and current management consists of maximum cytoreductive surgery to reduce tumor residuum to a minimum, followed by chemotherapy. Cytoreductive surgery appears to contravene traditional principles of cancer surgery because disease-free margins are rarely obtained. Prospective randomized studies to evaluate the benefit of primary maximum cytoreductive surgery have not been performed.

Both response to chemotherapy and survival are related to the volume of residual disease after surgery.[1-3] Patients with advanced disease debulked to a residuum of <1.5 cm diameter have the same prognosis as patients whose disease was <1.5 cm in diameter before intervention.[4] On the basis of these data, current surgical practice aims to leave the smallest residuum possible. However, the better prognosis of women with minimal residual disease may be due to their having less aggressive tumors rather than being an effect of the surgery itself. The different reported rates of maximum cytoreductive surgery vary widely, reflecting the determination and surgical experience of some and the scepticism regarding the need for such extensive surgery by others.

If maximum cytoreductive surgery is responsible for improving the prognosis in ovarian cancer, the median survival of the whole group should be greater in those series containing a high percentage of patients undergoing maximum cytoreductive surgery than in those series with a low rate.

Methods

Selection of studies. A literature search of *Medline* was performed for publications under the headings "ovarian cancer," "ovarian carcinoma" and "ovarian neoplasms," from 1976 to 1989, and in the *Index Medicus* under the heading "ovarian neoplasms" from 1967 to 1989. In addition, unpublished data from the third North Thames Ovarian Cancer Trial and from a multicenter study run by the Cancer Research Campaign Trial Office in Glasgow were included.

Studies were selected if they were composed of patients with predominantly stage III and stage IV ovarian cancer and if the overall median survival time and proportion of patients undergoing maximum cytoreductive surgery were shown. Many studies were unsuitable because median survival time was not stated for the group as a whole but only for patients who underwent successful surgery. The following was re-

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corded for each cohort: total number of patients, percentage of stage III cases, percentage of stage IV cases, percentage of maximum cytoreductive surgery, dose intensity of chemotherapy, median survival time, use of platinum-containing chemotherapy, and year of publication.

Maximum cytoreductive surgery was defined in terms of residual disease as follows: in 78.4% of the cohorts as <2 cm in diameter, in 5.2% as <3 cm, in 4.1% as <1.5 cm, in 2% as <1 cm, and in 10.3% by a verbal description such as "no palpable residual tumor."

Assessment of chemotherapy. To make allowance for chemotherapy regimens of differing effectiveness, all regimens were classified as either platinum-containing or non-platinum-containing. In addition, the potency of the chemotherapy was quantified as dose intensity because this method has already been shown to correlate with median survival time of patients with advanced ovarian cancer.[5] Calculation of dose intensity was only possible for regimens containing the following drugs, either singly or in combination: cisplatin, cyclophosphamide, hexamethylmelamine, doxorubicin, melphalan, and chlorambucil. Dose intensity was calculated as previously described.[5]

Statistical methods. The data are summarized in Table I. The effect on median survival time of the following six variables was investigated by multiple linear regression: percent stage III, percent stage IV, percent maximum cytoreductive surgery, dose intensity, year of publication, and whether the patients received platinum-containing chemotherapy. The regression was weighted by the number in each cohort, thus tending to give larger studies more influence than smaller ones. The variability of median survival time was found to increase with median survival time itself, which invalidates regression analysis. This was resolved by logarithmic transformation of the data, which is equivalent to looking at proportional rather than absolute changes in median survival time. Anscombe's test[60] was used to confirm objectively that this problem had been resolved. The deviations of the actual from the predicted median survival time were also found to have a normal distribution after logarithmic transformation, as confirmed by the Shapiro-Francia W′ test[61] and as required for the analysis. Results are quoted as two-sided p values and 95% confidence intervals, and p values <5% are described as statistically significant. The analysis was carried out using the Minitab software package.[62]

Results

A total of 58 separate studies, containing 97 cohorts and 6962 patients, was identified. Of the 97 cohorts, 76 could be ascribed a dose intensity. Without taking into account the possible effects of the chemotherapy used or the percent stage IV cases, there does seem to be an association between the percent maximum cytoreductive surgery and median survival (Fig. 1). Each 10-point increase in percent maximum cytoreductive surgery was associated with an estimated 16.3% increase in median survival time (95% confidence interval 10.4% to 22.5%, $p < 0.001$). A plot using only the cohorts for which dose intensity could be calculated gave a very similar regression, indicating that these 76 were very likely to be representative of the whole study population.

Multiple linear regression of log median survival time on percent stage IV, dose intensity, use of platinum, and percent maximum cytoreductive surgery was performed (Table II). Each 10-point increase in percent stage IV was associated with an estimated 2.6% decrease in median survival time (95% confidence interval 0.1% to 5.0%, $p = 0.038$). Each increase of 0.2 unit in dose intensity was associated with an estimated 11.1% increase in median survival time (95% confidence interval 6.0% to 17%, $p < 0.001$), and use of platinum was associated with an estimated increase of 53% in median survival time (95% confidence interval 35% to 73%, $p < 0.001$). Each 10-point increase in percent maximum cytoreductive surgery was associated with an estimated 4.1% increase in median survival time (95% confidence interval −0.63% to +9.1%, $p = 0.089$, Fig. 2). There is some evidence of increased percent maximum cytoreductive surgery being beneficial, independent of percent stage IV, dose intensity, and use of platinum, although the size of any effect is relatively small.

Interactions between variables and nonlinear effects were found not to be significant. In particular, there was no evidence that the size of the effect of platinum was dependent on percent maximum cytoreductive surgery ($p = 0.87$). The inclusion of percent stage III and year of publication, in addition to percent stage IV, dose intensity, platinum, and percent maximum cytoreductive surgery, had no significant effect on the analysis ($p = 0.82$ and 0.81, respectively).

Two cohorts had a much larger influence than the rest on this analysis, as measured by "leverage."[61, 62] These were both from reference 14 (Table I). Omitting them from the analysis slightly increases the effect of percent maximum cytoreductive surgery, each 10-point increase in which is then associated with an estimated 4.6% increase in median survival time (95% confidence interval −0.15% to +9.8%, $p = 0.058$).

If the effect of dose intensity is ignored and all 97 cohorts are included in the analysis, the effect of percent maximum cytoreductive surgery was statistically significant ($p = 0.014$) and each 10-point increase in it was associated with a 6.1% increase in median survival time (95% confidence interval 1.3% to 11.2%).

This analysis might be criticized for including all cohorts without regard to which study they were from.

Table I. Advanced ovarian cancer: Data obtained from literature search

No. of cases	*Stage III (%)*	*Stage IV (%)*	*Maximum cytoreductive surgery (%)*	*Dose intensity*	*Mean survival time (mo)*	*Year*	*Use of platinum*	*Reference*
107	100.0	0.0	33.6	0.000	8.0	1968	Not used	6
89	0.0	100.0	9.0	0.000	10.0	1967	Not used	7
35	100.0	0.0	45.7	0.000	19.0	1967	Not used	7
31	81.0	19.0	35.0	1.050	23.5	1983	Used	8
25	76.0	24.0	32.0	0.997	27.5	1983	Used	8
94	71.0	29.0	29.0	—	19.6	1984	Not used	9
92	68.5	31.5	32.0	1.100	30.7	1984	Used	9
45	100.0	0.0	42.2	—	14.0	1972	Not used	10
104	80.8	19.2	43.3	0.530	18.6	1981	Not used	11
65	75.4	16.9	53.8	1.150	32.0	1988	Used	12
41	80.5	19.5	20.0	—	29.0	1978	Not used	13
39	82.0	18.0	28.0	0.530	17.0	1978	Not used	13
640	100.0	0.0	25.0	0.530	15.0	1979	Not used	14
183	0.0	100.0	16.4	0.530	10.5	1979	Not used	14
54	79.6	20.4	31.5	1.000	15.0	1979	Not used	15
195	100.0	0.0	22.6	0.530	10.0	1983	Not used	16
57	0.0	100.0	7.0	0.530	7.0	1983	Not used	16
94	72.0	22.0	48.0	1.100	26.0	1987	Used	17
97	71.0	27.0	50.0	0.770	26.0	1987	Used	17
186	76.3	23.7	44.1	0.570	17.0	1986	Used	18
183	75.4	24.6	49.2	0.570	17.0	1986	Used	18
175	79.4	20.6	33.1	0.750	23.8	1987	Used	19
182	80.8	19.2	28.6	0.440	21.4	1987	Used	19
174	81.6	18.4	29.9	0.210	19.4	1987	Used	19
49	75.5	24.5	53.0	0.310	19.0	1985	Used	20
37	75.7	24.3	43.0	0.450	12.0	1985	Not used	20
57	78.9	12.3	43.9	0.900	22.0	1988	Used	–*
21	71.4	28.6	42.8	0.470	16.5	1982	Not used	21
21	66.7	33.3	38.1	0.750	40.0	1982	Used	21
20	90.0	10.0	10.0	0.690	18.0	1983	Used	22
37	81.1	18.9	10.0	1.020	25.0	1983	Used	22
131	76.3	19.8	42.7	0.620	10.0	1981	Not used	23
130	83.8	13.8	39.2	—	12.0	1981	Not used	23
108	100.0	0.0	60.0	—	27.0	1989	Not used	24
19	0.0	100.0	16.0	—	16.0	1989	Not used	24
43	100.0	0.0	14.0	0.625	16.5	1989	Not used	25
37	100.0	0.0	21.6	—	11.0	1989	Not used	25
45	100.0	0.0	28.9	0.700	13.0	1989	Used	25
49	100.0	0.0	32.7	0.700	21.0	1989	Used	25
57	100.0	0.0	50.9	0.700	21.0	1989	Used	25
28	17.9	82.1	14.3	—	10.0	1981	Not used	26
29	10.3	89.7	10.3	0.710	11.0	1981	Not used	26
57	93.0	7.0	30.0	0.910	23.0	1985	Not used	27
63	94.0	6.0	27.0	0.870	24.0	1985	Used	27
26	84.6	15.4	19.2	0.560	11.0	1983	Used	28
27	88.9	11.1	14.8	0.710	12.0	1983	Not used	28
35	68.6	31.4	2.9	0.870	19.0	1982	Used	29

*Harding M, Milsted R, Kaye S, et al. Long term results of cyclophosphamide, Adriamycin and platinum chemotherapy in advanced epithelial ovarian cancer. Unpublished observations.

This assumes that, after allowing for percent maximum cytoreductive surgery, percent stage IV, dose intensity, and platinum, two cohorts from different studies are as alike as two cohorts from the same study. To validate this assumption the analysis was repeated with the use of only the largest cohort from each study. The best model with the use of two predictor variables was that with dose intensity and platinum, and adding none of the remaining four predictor variables gave a significantly better fit. A 10-point increase in percent maximum cytoreductive surgery was associated with only a 2.8% increase in median survival time (95% confidence interval −2.5% to 8.1%, $p = 0.29$). When the effect of dose intensity was ignored and all 97 cohorts were used, the effects of percent maximum cytoreductive surgery, platinum, and year of publication were significant, the last presumably acting as a "proxy" for dose intensity, which has tended to increase over time. In this model a 10-point increase in percent maximum cytoreductive surgery was associated with a 5.9% increase in median survival time (95% confidence interval 0.1% to 11.4%, $p = 0.046$).

Table I—Cont'd

No. of cases	*Stage III (%)*	*Stage IV (%)*	*Maximum cytoreductive surgery (%)*	*Dose intensity*	*Mean survival time (mo)*	*Year*	*Use of platinum*	*Reference*
18	83.3	16.7	55.6	0.280	20.0	1981	Used	30
18	77.8	22.2	27.8	0.420	19.0	1981	Used	30
38	71.0	21.0	32.0	0.830	23.5	1983	Used	31
19	69.2	30.8	10.5	0.330	16.0	1982	Not used	32
17	76.9	23.1	11.8	0.630	17.5	1982	Used	32
190	62.0	38.0	31.0	0.400	10.4	1984	Not used	33
185	54.0	45.0	29.0	—	9.1	1984	Not used	33
61	83.6	16.4	34.4	0.790	42.5	1989	Used	34
118	83.1	16.9	38.2	0.600	25.3	1989	Used	34
50	78.0	22.0	50.0	0.460	27.0	1989	Used	35
40	92.5	7.5	27.5	—	16.0	1988	Not used	36
36	86.1	13.9	22.2	—	12.5	1988	Not used	37
55	87.0	13.0	36.0	1.000	45.0	1988	Used	37
74	77.8	12.2	44.3	0.860	26.4	1983	Not used	1
84	77.8	12.2	44.3	0.790	29.6	1983	Used	37
342	84.0	16.0	36.0	0.520	21.6	1988	Used	37
21	66.7	33.3	23.8	0.680	12.0	1986	Used	38
22	54.5	45.5	27.2	0.400	21.2	1981	Used	35
32	84.4	15.6	18.7	0.500	16.0	1982	Not used	40
20	60.0	40.0	55.0	—	20.0	1987	Used	41
52	88.5	11.5	35.0	0.630	37.0	1987	Used	42
45	64.0	36.0	26.7	1.120	28.0	1987	Used	43
135	78.5	21.5	25.9	0.420	21.0	1987	Used	44
132	77.2	22.8	24.2	0.680	25.6	1987	Used	45
89	77.5	22.5	56.2	0.610	24.6	1985	Used	45
92	80.4	19.6	54.3	0.700	24.6	1985	Used	46
48	68.8	31.2	29.2	0.520	22.0	1985	Used	46
31	81.0	19.0	35.0	1.200	27.5	1989	Used	47
25	76.0	24.0	32.0	0.980	24.3	1989	Used	48
37	73.0	27.0	32.4	0.690	18.0	1987	Used	48
40	75.0	25.0	87.0	0.790	48.0	1988	Used	49
28	100.0	0.0	17.9	—	23.5	1981	Not used	50
20	0.0	100.0	0.0	—	7.5	1981	Not used	51
62	71.0	29.0	24.0	—	19.0	1986	Used	51
39	84.0	16.0	56.0	0.740	21.0	1984	Used	52
36	63.9	33.3	33.3	—	14.0	1985	Used	53
88	75.0	25.0	63.6	0.740	24.0	1986	Used	54
34	100.0	0.0	38.0	0.300	14.0	1986	Used	55
27	100.0	0.0	26.0	0.420	24.0	1986	Used	55
11	0.0	100.0	18.0	0.300	14.0	1986	Used	55
19	0.0	100.0	15.8	0.420	14.0	1986	Used	55
42	71.4	28.6	28.6	0.940	13.0	1985	Used	56
43	67.4	32.6	18.6	0.310	11.0	1985	Not used	56
38	100.0	0.0	42.0	0.830	28.0	1988	Used	57
47	76.6	23.4	6.0	—	12.5	1985	Not used	58
49	75.5	24.5	8.0	0.540	12.5	1985	Not used	58
22	77.3	22.7	23.0	—	19.5	1985	Not used	58
21	81.0	19.0	38.0	—	19.5	1985	Not used	58
174	100.0	0.0	47.7	—	29.0	1989	Used	59
184	100.0	0.0	49.5	—	27.0	1989	Used	14

Measurement of the size of residual disease is often not accurate and the definition of maximum cytoreductive surgery varies. The effect of changing the definition was examined. In 12 cohorts some patients may have had residual disease >2 cm in diameter. When these were omitted, a 10-point increase in percent maximum cytoreductive surgery was associated with an estimated 5.8% increase in median survival time (95% confidence interval 0.9% to 11.0%, $p = 0.02$). In 21 of the cohorts the percentage of patients left with no residual disease whatsoever was stated. When the multiple linear regression was repeated on the 18 of these for which dose intensity could be calculated and with percent stage IV, dose intensity, platinum, and percent with no residual disease used as predictors, each 10-point increase in percent with no residual disease was associated with an estimated 6.9% increase in median survival time (95% confidence interval −16.0% to 36.0%, $p = 0.56$). This lack of significance could be due to the small number of cohorts, although this did not prevent the other three variables from reaching significance, the p values for percent stage IV, dose intensity, and platinum being

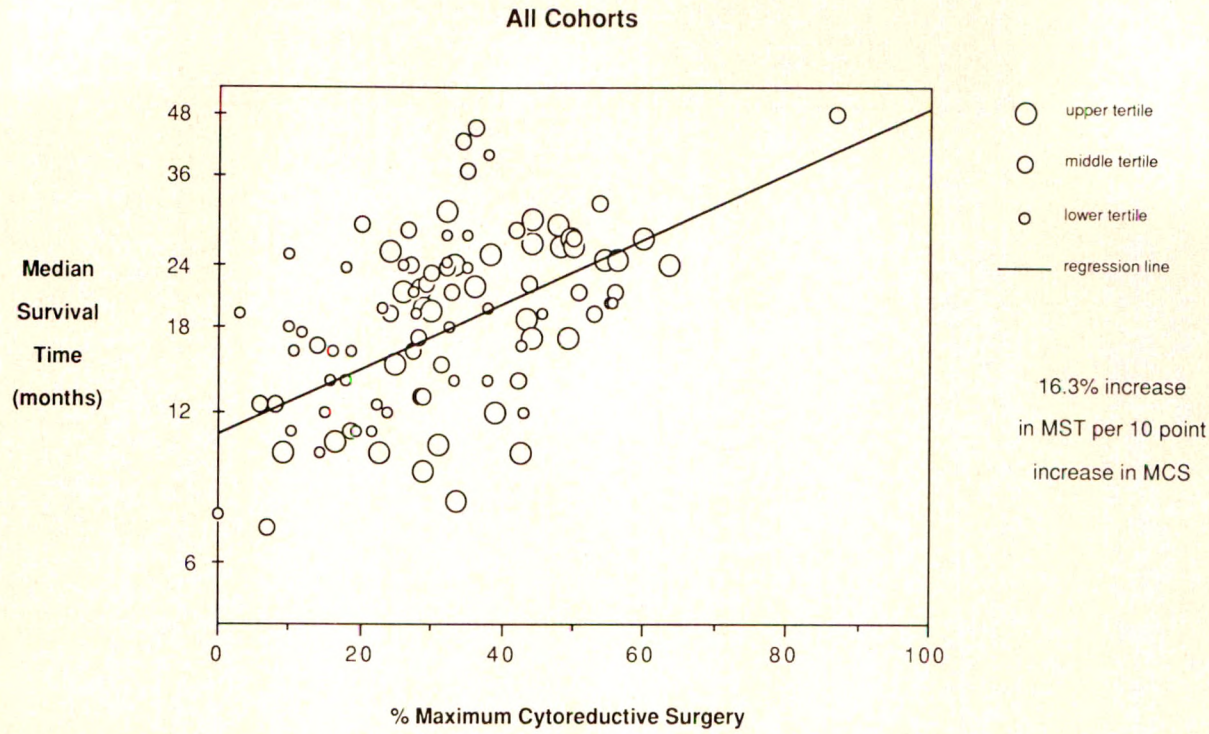


Fig. 1. Graph of log median survival time *(MST)* (months) versus percent maximum cytoreductive surgery *(MCS)* for all 97 cohorts. Effects of chemotherapy and percent stage IV cases are ignored.

Table II. Results of multiple linear regression analysis showing percent increase in median survival time, 95% confidence intervals, and p values associated with different variables

Variable	*Increase in mean survival time (%)*	*95% Confidence interval*	*Significance*
Use of platinum	53.0	35.0% to 73.0%	$p < 0.001$
Dose intensity (each 0.2 unit increase)	11.1	6.0% to 17.0%	$p < 0.001$
Patients with stage IV disease (%) (each 10-point increase)	−2.6	−0.1% to −5.4%	$p < 0.011$
Patients undergoing maximum cytoreductive surgery (%) (each 10-point increase)	4.1	−0.6 to 9.1%	$p = 0.089$

0.025, 0.012, and <0.001, respectively. This suggests that the effect of percent maximum cytoreductive surgery is still small regardless of the precise definition.

Comment

The results of this meta-analysis suggest that only a small improvement in median survival is achieved by maximum cytoreductive surgery in women with advanced ovarian cancer. In contrast, the use of platinum-containing chemotherapy appears to be associated with a much greater prolongation of median survival, confirming the results of Peto and Easton.[63] This substantial survival advantage after platinum may be because platinum agents were not available as second-line therapy in many of the studies. In this data set median survival time was also improved by increasing the dose intensity of chemotherapy, concurring with the findings of Levin and Hryniuk.[5] However, the dose intensity in the older studies in this analysis was very low, so this finding may be less relevant to modern practice.

That surgery should have such a small impact on survival is surprising in view of its well-established role in the primary treatment of advanced ovarian cancer and the importance placed on achieving a high percentage of maximum cytoreductive surgery.[24] The statistical significance of percent maximum cytoreductive surgery is sensitive to the inclusion or exclusion of a very small number of cohorts. However, whether these cohorts are or are not included, the size of any improvement associated with increased percent maximum

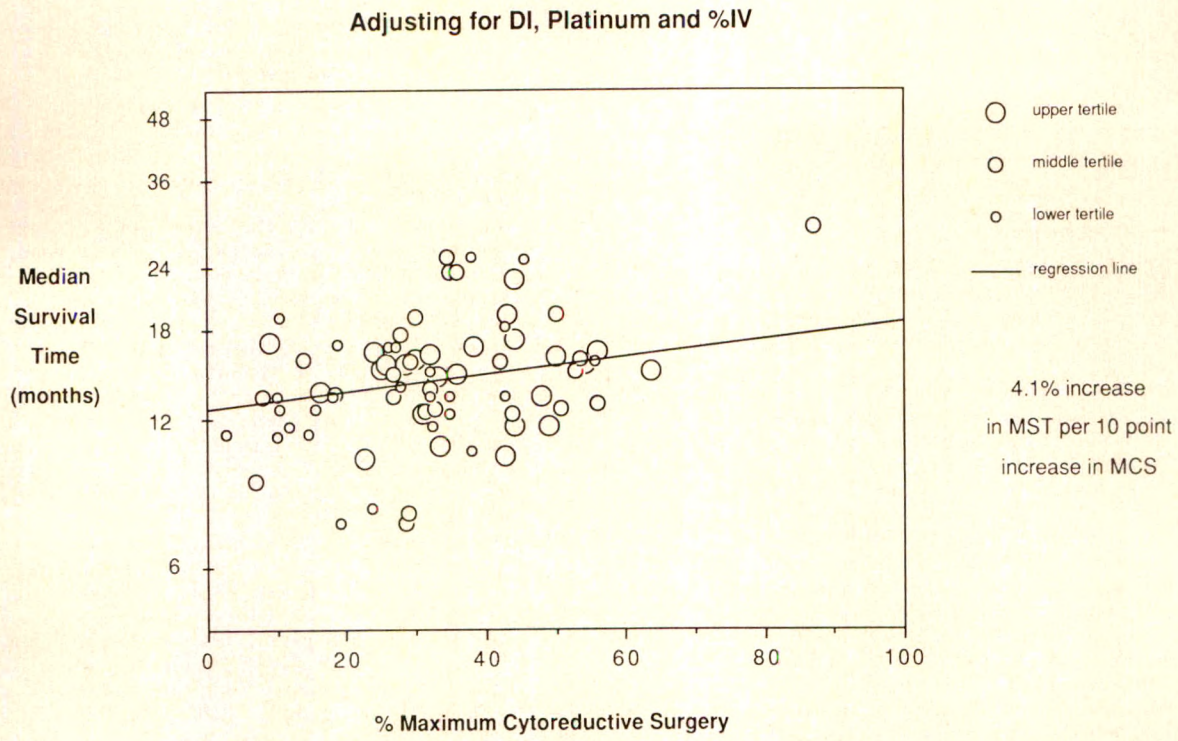


Fig. 2. Graph of log median survival time *(MST)* (months) versus percent maximum cytoreductive surgery *(MCS)* for 76 cohorts for which dose intensity could be calculated after adjustment for effects of dose intensity, percent stage IV, and platinum chemotherapy.

cytoreductive surgery is still relatively small, and this is probably more important than the existence or otherwise of formal statistical significance. Publication bias is unlikely to have affected the conclusions of this analysis, because the large majority of the studies included were investigating chemotherapy rather than surgery. Any publication bias that may exist would tend to exaggerate any beneficial effects of surgery.

The value of radical cytoreductive surgery in improving patient survival has been questioned by others. Hacker et al.[3] have shown that patients with metastatic disease >10 cm before surgery did poorly in spite of optimal cytoreduction, and Webb[24] found that patients in whom bowel resection was necessary to produce maximum cytoreductive surgery had a very poor prognosis. Patients requiring resection of the urinary tract also fare badly,[64] with a median survival time of 12 months, which is further reduced to 7 months if preoperative intravenous urography shows obstruction.

Several uncontrolled studies have shown that patients with minimal residual disease after surgery have a better survival and response to chemotherapy than those left with bulky residual disease.[1-3] However, it seems that using cytoreductive surgery to increase the proportion of such women in a population of patients with advanced ovarian cancer confers only a small survival advantage on the group as a whole. It seems likely that this is another example of the "Will Rogers phenomenon," in which reclassification of patients can improve the survival of the new subgroups without altering the survival of the whole population.[65]

Median survival is not the only measure of treatment benefit. This study could not address the effect of surgery on short-term survival and quality of life or on long-term survival. The symptoms of advanced ovarian cancer are relieved by excision of all large tumor masses. An improved quality of life after cytoreduction was seen in terms of returning to work, eating a normal diet, and enjoying life, compared with patients who had suboptimal surgery.[66] These women had less frequent hospital admissions, fewer procedures such as paracentesis, and fewer episodes of bowel obstruction. However, this was not a controlled study.

It is concluded that maximum cytoreduction surgery produces at best only a small improvement in median survival in advanced ovarian cancer. There may be other benefits such as relief of symptoms and improved quality of life. The use of bowel or urinary tract resection to debulk disease is more questionable because of the associated poor survival and higher morbidity. A large, controlled trial of surgery in advanced ovarian cancer is required to confirm or refute these conclu-

sions. However, there will be considerable practical and ethical difficulties in any such trial.

We thank the many colleagues who have helped by discussing this analysis or by providing invaluable information from their own studies.

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Transabdominal chorionic villus sampling: A modified freehand ultrasonographically guided technique

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A simple modification of the technique of transabdominal chorionic villus sampling is described. This technique uses a short piece of extension tubing between the needle hub and aspirating syringe. The advantages of this technique are discussed. (Am J Obstet Gynecol 1992;166:512.)

Key words: Chorionic villus sampling, ultrasonography

Transabdominal chorionic villus sampling is an effective method for prenatal diagnosis, allowing adequate sampling in >90% of cases.[1] In the typical two-person technique, an assistant performs the ultrasonographic examination. The primary operator inserts the biopsy needle into the placenta, connects a 20 ml syringe to the needle hub, and then aspirates during a slow, backward-and-forward movement of the needle tip.[2] However, the sudden aspiration that is required for tissue sampling may alter the position of the needle in the placenta. Furthermore, redirection of the needle during aspiration is cumbersome and does not readily permit fine needle placement. A modification of the transabdominal villus sampling technique allows the operator to concentrate on needle placement, while the assistant's primary responsibility is aspiration.

Method

Simultaneous ultrasonographic imaging is performed with a 3.5 MHz curvilinear transducer (General Electric model RT3200; General Electric, Milwaukee). A 15 cm rather than a 9 cm sampling needle provides the operator with sufficient needle length to obtain a villus sampling in nearly every patient.

Before the procedure is started, a 20 ml syringe containing 3 ml of transport medium is attached to a short piece of extension tubing (0.5 ml dead space) (Fig. 1).

Once the operator has inserted the needle into the placenta, the stylet is withdrawn and the distal end of the extension tubing is attached to the needle hub. Repeated rapid aspiration of the plunger is applied by an assistant to obtain a chorionic villus sample. The extension tubing removes the assistant from direct contact with the needle. The operator can therefore concentrate on the important tasks of scanning and needle localization. Continuous monitoring of the needle tip helps to avoid inadvertent amniotic membrane penetration.

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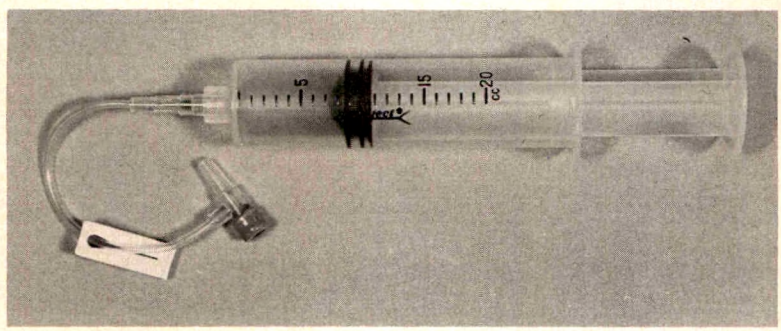


Fig. 1. Short piece of extension tubing (0.5 ml) is attached to syringe containing transport medium before chorionic villus sampling.

The Diabetes in Early Pregnancy Study: Changes in cholesterol, triglycerides, body weight, and blood pressure

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This study examined changes in cholesterol, triglycerides, body weight, and blood pressure during pregnancy in 312 diabetic and 356 control women recruited within 21 days after conception. Cholesterol values rose in both groups but were significantly lower in diabetic women at each time point (166 vs 178 mg/dl at week 12, $p = 0.0004$). Triglyceride values also rose in both groups. Triglyceride levels did not differ between groups up to week 8 of gestation, but by weeks 10 to 12 they were significantly lower in diabetic women than in controls (75 vs 89 mg/dl at week 12, $p = 0.0004$). Although they were no heavier at entry, diabetic women gained significantly more weight between weeks 6 and 8 ($p < 0.001$), resulting in a mean difference between groups of 1 kg. Systolic blood pressure increased steadily and significantly in the diabetic but not the control women (115.8 ± 16.2 SD vs 109.3 ± 11.8 mm Hg, $p = 0.0006$ at term). Diastolic blood pressure was higher in diabetic women on entry (70.7 vs 67.3 mm Hg, $p = 0.0006$) and throughout gestation. Significant correlations were found in the diabetic group between maternal blood pressure and lipids and infant birth weight. These newly found differences in cholesterol and triglyceride levels, weight gain, and blood pressure between type I diabetic and control women during gestation may have long-term cardiovascular implications. (Am J Obstet Gynecol 1992;165:513-8.)

Key words: Pregnancy, diabetes, lipids, blood pressure, weight

Although the outlook has improved, the diabetic woman and her pregnancy remain at higher risk during gestation, and the mother has a fivefold risk for a cardiovascular event during her lifetime compared with the nondiabetic woman.[1-9] Because the National Institute of Child Health and Human Development—Diabetes in Early Pregnancy Study recruited control and diabetic women before or within 21 days after conception, it provided a unique opportunity to study maternal cholesterol, triglycerides, blood pressure, and weight change in diabetic and control women throughout gestation. The results reported here show marked differences for these variables between diabetic and control women.

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Material and methods

The study included Cornell University, Harvard University, Northwestern University, The University of Pittsburgh, and The University of Washington. The National Institute of Child Health and Human Development was the data and coordinating center. The study design has been described in detail elsewhere.[10, 11] When the diagnosis of pregnancy was made during the week of the missed menses, diabetic subjects were hospitalized for 2 to 5 days for monitoring of metabolic control and performance of entry testing. Other than reinforcing the techniques of self–blood glucose monitoring, a standard approach to the management of diabetes was not imposed nor were fixed goals for glycemic control mandated. After discharge, diabetic subjects were monitored at weekly clinic visits from weeks 5 through 12 after the last menstrual period and monthly thereafter. Control subjects were entered and followed at biweekly intervals. Controls were screened for gestational diabetes at 26 weeks according to the criteria established by The Second International Gestational Diabetes Workshop.[12]

The thiobarbituric acid method was used for glycosylated hemoglobin analysis.[13] This method is not affected by fetal hemoglobin, which made it possible to perform all the tests on frozen, washed red blood cells

Table I. Maternal variables (mean ± SD)

	Diabetic (n = 312)	*Control (n = 356)*	*Significance*
Height (cm)	163.2 ± 7.3	164.1 ± 7.4	$p = 0.13$
Age (yr)	27.9 ± 4.0	29.6 ± 3.7	$p < 0.0001$

in a single laboratory. The method relies on the formation of a color when the Amadori rearrangement product (1-deoxy-fructosyl adduct) is heated in the presence of 0.3 oxalic acid to form 5-hydroxymethylfurfuraldehyde. Laboratory plasma glucose measurements were performed in each center by an enzymatic method.

Blood pressure was measured by standard protocol.[14] Plasma cholesterol and triglyceride levels were measured at each institution by standard methods.[15-17] Only two patients were on oral contraceptives in the month before conception.

Associations were measured by Fearson correlations, and comparisons were by t tests and paired t tests. Statistical significance was declared at the two-sided 0.05 level. Data were derived as repeated measures during the course of gestation, but a univariate analysis (by two-sided t test) at the examined weeks was made to better view weekly differences. Since there were at most six such comparisons, a Bonferroni criterion for statistical significance was applied to address the multiple comparisons problem presented by this approach. Thus, if a variable were measured at six different weeks of gestation, then a comparison at any week was judged significant at the 0.05 level if its p value did not exceed $0.05/6 = 0.0083$. In assessing whether a decreasing or increasing trend was present in the serial data, it was noted that observed changes were all monotone, so that an adequate test for trend was the paired t test of last measurement minus first measurement. Associations were measured by Pearson correlation coefficients.[10, 11, 18-21]

Results

The analysis was performed on 312 diabetic and 356 control patients who were delivered of live infants. Table I shows the comparison between diabetic and control groups for maternal height and age at entry into the study. The diabetic and control mothers were not different for height, but the control mothers were older. Table II documents the glycosylated hemoglobin by gestational week for each group. As would be expected, significant ($p = 0.0005$) differences were present between groups at each time point.

Table III shows the cholesterol values for the two groups of women during weeks 6 to 12 of gestation. The difference was most pronounced at 12 weeks with diabetic women having a mean cholesterol level of 165.7 ± 29.5 SD and control women having a mean level of 177.8 ± 36.0 mg/dl. The mean cholesterol level in the controls was significantly ($p < 0.01$) higher at each time point. Cholesterol and glycosylated hemoglobin values were not significantly correlated. A trend toward rising levels of cholesterol was seen in both diabetic and control populations and was significant when week 12 was compared with week 6 ($p = 0.0001$ for both groups). A significant correlation in the diabetic group was found between change in cholesterol levels between weeks 6 and 8 and infant birth weight ($p = 0.03$).

Table IV compares triglyceride levels in diabetic and control subjects. The two groups did not differ significantly at week 6 or 8. By week 12, the triglyceride levels of the diabetic women were significantly ($p = 0.0004$) lower than those seen in the controls (75.1 ± 36.2 SD vs 89.4 ± 44.4 mg/dl). Triglyceride levels rose significantly ($p < 0.001$) in both diabetic and control groups from week 6 to week 12. A correlation was not found between triglyceride levels and glycosylated hemoglobin values nor between triglyceride and cholesterol levels. A significant correlation in the diabetic group was found between change in triglyceride levels between weeks 6 to 10 and infant birth weight.

Table V documents the weights and Table VI the body mass index in the two groups of women throughout gestation. The groups did not differ significantly at initial hospitalization or at week 6. By week 8 the diabetic group was heavier and had a higher body mass index at each later comparison point until week 36. The change in weight and percent change began to be significantly higher in the diabetic group by week 8 ($p < 0.001$, week 8 vs week 6 between groups) and continued to be so at each later comparison point. The diabetic group reached term having gained 15 kg of body weight versus 14 kg for the control group. Tables VII through IX compare systolic, diastolic, and mean blood pressure in the two groups. The diabetic subjects had significantly higher systolic blood pressure at entry and from week 12 of gestation onward. At week 6, systolic blood pressure was not different between the groups (107.0 ± 11.4 SD for the control group and 108.7 ± 13.8 mm Hg for the diabetic group). Diastolic blood pressure was higher in the diabetic women at each time point, and after 20 weeks the diastolic pressure in the control women was about 5 mm Hg lower than in the diabetic group. For control women, systolic and diastolic blood pressures were lower at 6 weeks gestation than at entry and gradually rose to entry levels by delivery. In diabetic women, systolic blood pressure dropped significantly at 6 weeks but returned to entry levels by 12 weeks of gestation and continued to rise thereafter to term. Thus the change in blood pressure

Table II. Glycosylated hemoglobin (nanomoles of fructose per 100 gm hemoglobin) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
6	52.2 ± 13.1 (n = 206)	p = 0.0005	34.8 ± 5.6 (n = 228)
8	52.2 ± 12.8 (n = 278)	p = 0.0005	35.4 ± 6.0 (n = 285)
10	49.1 ± 11.2 (n = 276)	p = 0.0005	35.5 ± 6.5 (n = 281)
12	46.4 ± 10.2 (n = 278)	p = 0.0005	35.8 ± 6.3 (n = 277)
14	44.9 ± 9.0 (n = 179)	p = 0.0005	35.6 ± 6.0 (n = 242)

Table III. Cholesterol levels (milligrams per deciliter) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
6	150.5 ± 26.7 (n = 212)	p = 0.011	158.0 ± 27.5 (n = 283)
8	154.5 ± 30.2 (n = 256)	p = 0.013	161.6 ± 27.8 (n = 318)
10	157.7 ± 29.6 (n = 250)	p = 0.0004	168.4 ± 28.9 (n = 302)
12	165.7 ± 29.5 (n = 257)	p = 0.0004	177.8 ± 36.0 (n = 322)

Week 6 versus week 12, p = 0.0001 and p = 0.0001.

Table IV. Triglyceride levels (milligrams per deciliter) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
6	64.8 ± 25.7 (n = 212)	NS	63.8 ± 35.9 (n = 283)
8	65.8 ± 35.3 (n = 254)	NS	71.2 ± 28.7 (n = 318)
10	67.4 ± 33.0 (n = 250)	p = 0.0004	78.3 ± 31.6 (n = 301)
12	75.1 ± 36.2 (n = 256)	p = 0.0004	89.4 ± 44.4 (n = 322)

NS, Not significant. Week 6 versus week 12, p = 0.0001 and p = 0.0001.

Table V. Weight (kilograms) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
Entry	62.9 ± 9.8 (n = 310)	NS	62.8 ± 12.6 (n = 355)
6	63.4 ± 9.6 (n = 218)	NS	63.5 ± 13.2 (n = 284)
8	64.7 ± 9.4* (n = 269)	NS	63.7 ± 13.1 (n = 321)
10	65.3 ± 9.4 (n = 255)	NS	64.4 ± 13.4 (n = 296)
12	66.2 ± 9.8 (n = 267)	NS	64.9 ± 13.3 (n = 321)
20	69.9 ± 9.8 (n = 249)	NS	68.2 ± 12.7 (n = 299)
28	73.9 ± 10.1 (n = 245)	NS	72.7 ± 13.5 (n = 295)
36	77.6 ± 10.0 (n = 166)	NS	76.5 ± 13.7 (n = 275)

NS, Not significant.
*Significant weight gain versus control ($p < 0.001$).

from the initial hospitalization to the later points is also significantly greater for the diabetic subjects. Mean blood pressure was significantly higher for the diabetic group at every time point. Infant birth weight correlated with maternal blood pressure in the diabetic group at the initial hospitalization (p = 0.01) and at 28 weeks (p = 0.03).

Comment

One of the objectives of the Diabetes in Early Pregnancy Study was to examine the effect of diabetes on cholesterol, triglycerides, body weight, and blood pressure during pregnancy. Our data indicate that there are significant differences between diabetic and control women in maternal cholesterol, triglycerides, weight and blood pressure, variables that might contribute to risk for the pregnancy or subsequent maternal vascular disease. Although no relationship was found between the variables under consideration and fetal loss or congenital malformations, a relationship was found between maternal blood pressure and lipid changes and infant birth weight.

In nonpregnant subjects, previous studies have reported elevated cholesterol and triglyceride levels. The degree of elevation was positively correlated with glycosylated hemoglobin values.[21] It has been reported that nonpregnant women with type I diabetes mellitus with a mean fasting glucose of 208 mg/dl and an av-

Table VI. Body mass index (Weight in kilograms/Height^2 in meters) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
Entry	23.6 ± 3.4 (n = 307)	NS	23.2 ± 4.1 (n = 345)
6	23.8 ± 3.2 (n = 215)	NS	23.5 ± 4.3 (n = 276)
8	24.3 ± 3.2* (n = 266)	p = 0.04	23.7 ± 4.3 (n = 314)
10	24.5 ± 3.2 (n = 251)	p = 0.04	23.9 ± 4.2 (n = 291)
12	24.8 ± 3.1 (n = 262)	p = 0.03	24.1 ± 4.4 (n = 313)
20	26.2 ± 3.3 (n = 246)	p = 0.002	25.3 ± 4.0 (n = 290)
28	27.8 ± 3.6 (n = 242)	p = 0.03	27.0 ± 4.4 (n = 286)
36	28.9 ± 3.1 (n = 164)	NS	28.4 ± 4.4 (n = 265)

NS, Not significant.
*Significant weight gain versus control ($p < 0.001$).

Table VII. Systolic blood pressure (millimeters of mercury) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
Entry	111.4 ± 9.9 (n = 275)	p = 0.005	108.4 ± 11.8 (n = 321)
6	108.7 ± 13.8 (n = 208)	NS	107.0 ± 11.4 (n = 270)
12	111.7 ± 14.6 (n = 262)	p = 0.0006	106.3 ± 11.4 (n = 315)
20	112.3 ± 15.3 (n = 246)	p = 0.0006	104.8 ± 11.4 (n = 295)
28	114.7 ± 16.3 (n = 239)	p = 0.0006	107.1 ± 11.6 (n = 298)
36	115.8 ± 16.2 (n = 169)	p = 0.0006	109.3 ± 11.8 (n = 281)

NS, Not significant.

erage age of 34 years had a 32 mg/dl greater total plasma triglyceride level and a 38 mg/dl greater total cholesterol level than nondiabetic women of similar age.[22]

Studies of pregnant diabetic patients have found either no change or an increase in triglyceride content or cholesterol over controls. The sutdy that included and separated type I subjects from controls, type II subjects, and those with gestational diabetes by trimester did not detect differences between normal and type I subjects.[23] From these observations in small numbers of nonpregnant and pregnant diabetic women, we would have expected normal or elevated triglyceride and cholesterol levels in pregnant diabetic women and a positive correlation with glycosylated hemoglobin. We found the reverse trend in pregnant subjects. In fact, significantly lower levels were found in type I diabetic women in our study for cholesterol and for triglycerides after 8 weeks gestation; neither triglyceride nor cholesterol levels correlated with glycosylated hemoglobin. The reasons for the different findings in our study may be in part due to the large numbers of subjects studied, which allowed for definition of relatively small differences and the different treatment programs used.

Hormonal differences might also contribute to the observed differences in lipid levels. Significant correlations between sex hormones in healthy young women and plasma lipoprotein levels have been documented, and diabetic women have been shown to have significant differences in hormonal profiles during gestation (which tend to normalize if glucose control is improved) compared with nondiabetic controls.[8, 24, 25]

Our results suggest that disposal of triglyceride and cholesterol may differ between diabetic and control women. Diabetic subjects from one of the participating centers were found to have a reduction in high-density lipoprotein, particularly high-density lipoprotein 3.[26] Changes in lipid transport to the fetus could provide a potent stimulus to macrosomia, a morbid complication of diabetic pregnancy frequently cited in the literature in "well controlled" patients.[27-30] The significant correlations found between changes in cholesterol levels and triglyceride levels between weeks 6 and 10 and infant birth weight are consistent with this hypothesis.

The reasons for increased weight gain in the pregnant diabetic women between weeks 6 and 8 remain to be defined. Since weight in the two groups was the same at the beginning of gestation, it would appear that the increased weight gain seen in insulin-dependent diabetic women is the result of the treatment program or pregnancy per se. Increased caloric intake or fluid retention could both play a role. The possibilities that diabetic women receive different caloric advice, or excess caloric intake is required to prevent symptomatic hypoglycemia early in gestation are also potential explanations for the excess weight gain, with implications for the current protocols used to treat pregnant diabetic women. The Diabetes Control and Complications Trial also documented an excessive weight gain in those type

Table VIII. Diastolic blood pressure (millimeters of mercury) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
Entry	70.7 ± 7.3 (n = 275)	p = 0.0006	67.3 ± 8.8 (n = 321)
6	67.4 ± 10.2 (n = 208)	p = 0.08	65.2 ± 8.4 (n = 270)
12	67.7 ± 9.1 (n = 261)	p = 0.025	65.6 ± 8.3 (n = 315)
20	68.1 ± 15.9 (n = 246)	p = 0.0006	63.1 ± 8.2 (n = 295)
28	68.9 ± 10.3 (n = 239)	p = 0.0006	63.6 ± 8.7 (n = 298)
36	71.7 ± 10.2 (n = 169)	p = 0.0006	67.2 ± 9.0 (n = 281)

Table IX. Mean blood pressure (millimeters of mercury) (mean ± SD)

Week No.	*Diabetic women*	*Significance*	*Control women*
Entry	91.0 ± 7.9 (n = 275)	p = 0.0001	87.9 ± 9.2 (n = 321)
6	88.1 ± 10.9 (n = 208)	p = 0.04	86.1 ± 8.9 (n = 270)
12	89.8 ± 10.6 (n = 261)	p = 0.0001	86.0 ± 8.9 (n = 315)
20	90.2 ± 12.9 (n = 246)	p = 0.0001	83.9 ± 8.9 (n = 295)
28	91.8 ± 12.1 (n = 239)	p = 0.0001	85.4 ± 9.4 (n = 298)
36	93.7 ± 12.2 (n = 169)	p = 0.0001	88.3 ± 9.7 (n = 281)

I diabetic women who were in the intensive management treatment group.[31] Protocols that use carefully titrated insulin dosage and food intake in type I diabetic women throughout gestation have not necessarily been associated with weight gain >12 kg.[8] The potentially adverse health implications of this documented weight gain for the mother warrant further study.

Insulin administration has been associated with salt and water retention.[32, 33] It can be assumed that all diabetic women in the study had high circulating insulin levels, because all received therapeutic insulin administered subcutaneously with a mean dose of 1.08 U/kg/24 hours at term. High circulating insulin levels have been associated not only with a higher risk for vascular disease but also with hypertension.[34]

Diabetic women were found to have higher diastolic and systolic pressures at every time point during gestation except 6 weeks, when systolic blood pressure was similar in the two groups. The present data not only point to the difference in blood pressure between the two groups but also provide impetus for the reevaluation of norms regarding blood pressure and the diagnosis of hypertension during gestation. For example, a blood pressure of 140/90 mm Hg was at 3 SDs above the normal mean at any observation point during gestation in the control group. The coefficient of variation for systolic blood pressure was extremely stable in the control group but increased throughout gestation in the diabetic women, indicating a subset of women whose pressor response to gestation is different from controls. Future research should consider whether such pressor changes are the result of vascular volume differences or increased insulin levels, whether they identify women at risk for vascular disease, and whether they warrant treatment.

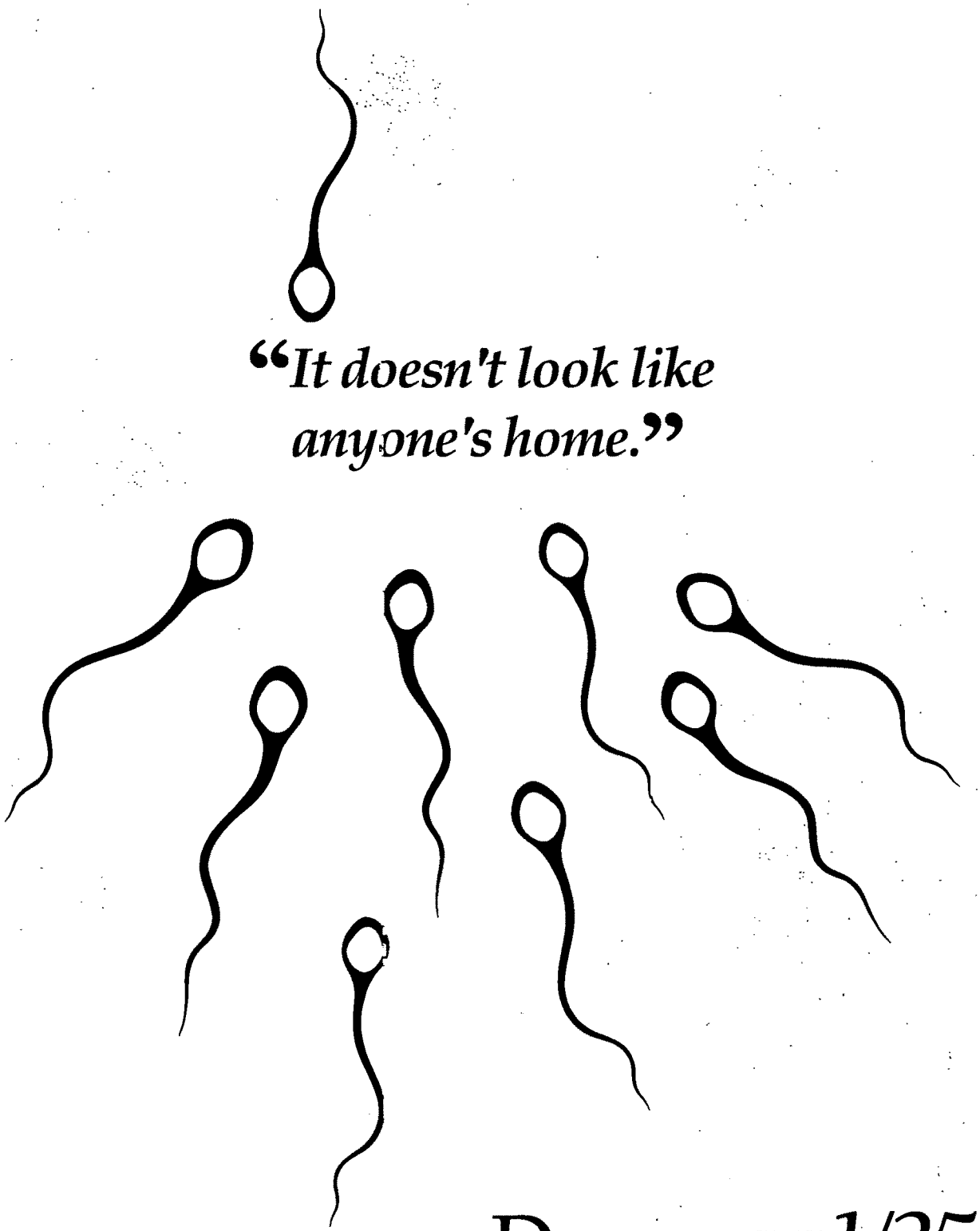
The present study demonstrates the need for long-term studies to determine whether changes in lipids, weight, and blood pressure noted during pregnancy persist post partum and if pregnancy influences risk for cardiovascular events in type I diabetic women. Our findings emphasize the need for studies during pregnancy and thereafter to develop standards related to outcome criteria and norms for risk factors for vascular disease in this population. For example, reference values for lipoprotein lipids have been described for 36 weeks' gestation[35] but not earlier in pregnancy. Such data might allow the identification of women at risk for vascular disease at a time in their lives when therapeutic intervention is highly feasible.

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1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women-Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. Latter group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myocardial ischemia, occurs with high progestational activity. Consider amount of both steroids in choosing OC. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age: <30	30-39	≥40
Heavy smokers (≥15/day)	C	B	A
Light smokers (<15/day)	D	C	B
Nonsmokers, no predispositions	D	C,D	C
Nonsmokers, with predispositions	C	C,B	B,A

Be alert to earliest manifestations of TE disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. 2. *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endo cancer with combination or progestogen-only OCs. Studies show no increased breast cancer with OCs or estrogens. H greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estroge and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with ot factors and in various subgroups. Decrease in benign breast tumors in OC users is well known. Some studies su increased risk of cervical dysplasia, CIN, erosion, and carcinoma in long-term users. Cervical microglandular hyperpl bean reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OCs surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfu causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history o cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dy 4. *Hepatic adenomas, etc,* have been associated with OCs. In 1 study OC formulations with high "hormonal potenc associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemoperi in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long-t users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdomin tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Ra and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may be Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur with 5. *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring:* Estrogens or progestogens duri pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens ma increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. Fr 90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES-e daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACT vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by males was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal wit pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardio defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,000 compared to 7.8 for those unexposed (P<.05). Female hormone exposure in utero was associated with testicular and cancer. More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were u threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence proge are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregna post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after s OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstrate out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and v OCs. If pregnancy is confirmed, stop OC and apprise patient of potential fetal risks and discuss continuation of pre Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnanc 6. *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 s appeared in 6-12 months' use in another. Bile duct cancer has been reported. 7. *Carbohydrate and lipid metabolism:* De glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglyc total phospholipids, and lipoproteins may increase. 8. *Elevated blood pressure* (BP) occurs in some users in a few and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but in with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension in Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pre familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during me cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardiov disease or stroke. High BP may or may not persist after stopping OCs. 9. *Headache:* Onset or exacerbation of migr development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating 10. *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinu BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleedi out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenor secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing, with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonge occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been ass with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in p with postpill amenorrhea when galactorrhea was present. 11. *Ectopics:* In failures with combination OCs, the ratio of to intrauterine pregnancies is no higher than in nonusers. 12. *Breast feeding:* OCs may decrease quantity and quality A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after w 13. *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable dif in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precaution** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvi BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor c in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy in risk of recurrence on OCs: observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysf and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megalo anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise path of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after st for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; inc prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; inc thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, unalter T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, trigly phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask o climacteric. Cervical chlamydial and gonorrheal prevalence is increased; do not assume OC protects against chlamydi HIV seropositivity was associated with OC use. *Information for patients.* See patient labeling. *Drug interactions.* OC effecti may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramph sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, anal tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, t antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetamin **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombop and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; c thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. T evidence of association between the following and OCs, though confirmatory studies have not been done; mes thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during an use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlarg secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, chol jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, cardiopathy, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refut premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, pares nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multifo nodosum, hemorrhagic eruption, herpes gestationis, hidradenitis suppurativa, hemolytic uremia, malignant hyperte impaired renal function, acute renal failure, sometimes irreversible, itching, vaginitis, porphyria, PVCs, abnormal pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythema rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant mela endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been reporte large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Co prescribing information should be consulted prior to use and is available on request.**

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Pelvic inflammatory disease: Findings during inpatient treatment of clinically severe, laparoscopy-documented disease

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OBJECTIVES: We evaluated the relationship between clinically severe pelvic inflammatory disease and laparoscopic diagnosis and grading, comparative treatment with clindamycin plus cefamandole or doxycycline, and a management protocol for inpatient pelvic inflammatory disease treatment.
STUDY DESIGN: Thirty-three patients who met our clinical criteria for severe pelvic inflammatory disease underwent diagnostic laparoscopy. Pelvic inflammatory disease patients were randomized to double-blind treatment with clindamycin plus cefamandole or doxycycline within our management protocol; postdischarge oral antibiotics were omitted.
RESULTS: Laparoscopy confirmed pelvic inflammatory disease in 23 (70%) patients; 10 (44%) had mild pelvic inflammatory disease by laparoscopic grading. Laparoscopic grade alone predicted necessary duration of therapy to response: mild pelvic inflammatory disease, 2.3 ± 0.5 days; moderate pelvic inflammatory disease, 2.7 ± 1.5 days; and severe pelvic inflammatory disease, 3.9 ± 1.5 days ($p < 0.05$). Using the management plan presented, response rates for both antibiotic regimens were 100%.
CONCLUSIONS: Clinical diagnosis and grading of severe pelvic inflammatory disease has poor specificity. Laparoscopic grading of severity of pelvic inflammatory disease seems accurate. Both clindamycin plus cefamandole and clindamycin plus doxycycline are equally effective regimens for treatment of pelvic inflammatory disease and did not require supplementation after discharge. Our management plan is objective and practical; daily bimanual examination is the most sensitive indicator of persistent disease.
(Am J Obstet Gynecol 1992;166:519-24.)

Key words: Pelvic inflammatory disease, laparoscopy, clindamycin

Laparoscopic examination of patients having pelvic inflammatory disease of unclassified severity by clinical diagnosis has shown the specificity of clinical diagnosis to be ≤65%.[1, 2] Clinically severe pelvic inflammatory disease, generally treated with inpatient therapy, is a diagnosis of uncertain specificity. In 1986 Washington et al.[3] estimated the direct costs of outpatient and inpatient treatment for pelvic inflammatory disease to be $150 and $2865, respectively, and patients with clinically more severe disease are in greater jeopardy from misdiagnosis. Thus for both economic and medical reasons a better understanding of the accuracy of a clinical diagnosis of severe pelvic inflammatory disease is important. Further, a well-accepted laparoscopic grading system[4, 5] for pelvic inflammatory disease is in place, but neither the scope of its usefulness in managing clinical disease nor its correlation with clinical findings has been fully explored.

Indications for inpatient treatment of pelvic inflammatory disease remain subjective and variable. Current recommendations for hospitalization from the Centers for Disease Control[6] are based on compliance issues and clinical severity of disease, but the latter is undefined. Further, after hospitalization and initiation of a parenteral antibiotic regimen, those circumstances that confirm that adequate therapy has been given and those indicating therapeutic failure with the regimen in use are not well understood. Although many antibiotic regimens have been used with success in the parenteral treatment of pelvic inflammatory disease, optimal therapy can be identified only with continued study of currently available agents.

We undertook this study to examine the accuracy of routine clinical diagnosis of pelvic inflammatory disease and our own criteria for severe pelvic inflammatory disease requiring inpatient therapy, to evaluate the clinical usefulness of the accepted laparoscopic grading system and our management plan (outcome classification system) defining therapeutic success and failure, and to study the relative efficacy and safety of clindamycin with either cefamandole or doxycycline in the treatment of clinically severe pelvic inflammatory disease.

Material and methods

Women seen in the outpatient and emergency facilities of Duke University Medical Center and assigned a clinical diagnosis of severe pelvic inflammatory disease, for which we uniformly use inpatient therapy, by an attending physician in the Department of Obstetrics and Gynecology were considered for this study. In that

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Table I. Therapeutic outcome classifications for patients hospitalized for pelvic inflammatory disease

Response: Requires at least five of the following
1. All symptom parameters (see text) = none or mild
2. Cervical motion and adnexal tenderness = none or mild
3. Abdominal tenderness and distention = none or mild
4. Temperature ≤37.8° C (oral) for ≥24 hr
5. White blood cell count between 4000/mm³ and 10,000/mm³ or >30% decrease if >10,000/mm³ on admission
6. Pelvic mass did not develop and if initially present did not increase in size on examination

Failure:

Progression of disease: After 24 hours of therapy, worsening of at least four of the parameters above; after 48 hours, at least three; onset of septic shock at any time

Inadequate improvement: After 96 hours of therapy, failure to improve in at least two of the parameters above

Late failure: Response criteria not met by 14 days of therapy or relapse of disease within 10 days of discontinuation of therapy (relapse defined as failure to continue to satisfy criteria for response above)

Side effect failure: Onset of any drug-related condition significantly threatening the comfort or health of the patient

pelvic inflammatory disease were required. However, to qualify for severe pelvic inflammatory disease and entry into this study, one or more of the following criteria were required: significant peritonitis defined by low abdominal rebound tenderness extending to the upper abdomen, vomiting, or paralytic ileus by examination; temperature ≥39.0° C orally; peripheral white blood cell count >20,000/mm³ or <4,000/mm³; postural hypotension; adnexal fixation or mass on examination; or persistence or progression of disease by objective criteria after ≥72 hours of outpatient antibiotic therapy. Those excluded from study were as follows: (1) patients who were allergic to clindamycin, doxycycline, or cephalosporins; (2) those who were pregnant or breast-feeding; (3) patients in septic shock; (4) those requiring other antibiotic therapy; (5) those having severe intercurrent medical disease; (6) those having pregnancy-related pelvic infection; (7) those previously entered in this study; or (8) those <12 years old. Written informed consent approved by the institutional review board was obtained from each patient before participation.

A detailed history, examination, and laboratory data were obtained by one investigator (C.H.L.), who also participated in all laparoscopy procedures and examinations during therapy. Patients were taken to the operating room, and a laparoscopic diagnosis was determined while they were under general anesthesia. Patients without erythema, edema, or grossly purulent exudate from the oviducts were categorized as not having pelvic inflammatory disease. Patients confirmed to have pelvic inflammatory disease were classified as having mild disease (any erythema or edema of freely movable oviducts without purulent exudate), moderate disease (significant erythema or edema of oviducts with any tubal fixation, patency uncertain), or severe disease (presence of an inflammatory mass).[4,5] While in surgery all patients had endocervical specimens taken for cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Patients with a laparoscopic diagnosis of pelvic inflammatory disease had oviduct specimens obtained by either endotubal swabbing at the fimbria or needle aspirate as available; these were cultured for *N. gonorrhoeae, C. trachomatis*, genital mycoplasmas, and other aerobic and anaerobic bacteria with special media and glove box techniques we have previously detailed.[7,8]

Patients with pelvic inflammatory disease were then randomized postoperatively to intravenous antibiotic therapy consisting of clindamycin, 600 mg every 6 hours, and either cefamandole, 2 gm every 6 hours, or doxycycline, 100 mg, plus 10 ml 4% sodium bicarbonate alternating with placebo (5% dextrose in water) every 6 hours to achieve double blinding.

At the time of study entry and at 24-hour intervals after initiation of antibiotic therapy, the following signs were assessed and graded as 0 (none), 1 (mild), 2 (moderate), or 3 (severe): direct and rebound abdominal tenderness, abdominal distention, cervical motion tenderness, left and right adnexal tenderness, and left and right adnexal enlargement. Symptoms were graded on the same scale: pain, anorexia, malaise, nausea or vomiting, and constipation or diarrhea. Thus at each assessment a total sign score (0 to 24), total symptom score (0 to 15), peak oral temperature during the preceding 24 hours, and the peripheral white blood cell count for that day were recorded. Outcome classifications were based on these data, as shown in Table I. Protocol antibiotic therapy was continued for 48 hours after declaration of response and for a minimum of 5 days (20 doses), after which patients were discharged without supplemental oral antibiotic treatment. They were given instructions to report any symptoms suggestive of relapsing disease, cards requesting notification of the investigators if they were seen by another physician for relapsing disease, a return appointment in 1 month, and instructions for presumptive treatment of all sexual partners. Patients were contacted by telephone

Table II. Selected characteristics of patients given a clinical diagnosis of clinically severe pelvic inflammatory disease, compared by laparoscopic diagnosis; examination, and laboratory data*

Variable	*Pelvic inflammatory disease absent (n = 10)*	*Pelvic inflammatory disease present (n = 23)*	*Significance*
Examination findings at presentation			
Grossly purulent vaginal discharge	6 (60%)	14 (61%)	NS
Oral temperature ≥38.0° C	2 (20%)	11 (43%)	NS
Total sign score (mean)	11.3 ± 4.9	15.9 ± 2.4	$p < 0.05$
Pelvic mass by examination	3 (30%)	7 (30%)	NS
Abdominal tenderness = severe	7 (70%)	22 (96%)	$p < 0.05$
Adnexal tenderness = severe	9 (90%)	23 (100%)	NS
Laboratory data at presentation			
White blood cell count ≥10,000/mm³	6 (60%)	14 (61%)	NS
Erythrocyte sedimentation rate >15 mm/hr	3/6 (50%)	15/18 (83%)	NS
Band cells (%)	1.8 ± 2.3	3.3 ± 4.9	NS

NS, Not significant.

*Data are given as frequencies with percents for the group or as mean values ± SD; different denominators resulting from missing data are indicated for the variables.

or personally 10 days after discharge for a status check.

Evaluability of treatment efficacy required that all protocol procedures and conditions were satisfied and that no other antibacterial therapy was given during the 10 days after completion of protocol therapy. Statistical tests used were the two-tailed *t* test, Mantel-Haenszel χ^2, and analysis of variance.

Results

Of the 33 patients given a clinical diagnosis of severe pelvic inflammatory disease by our criteria, laparoscopy confirmed the presence of pelvic inflammatory disease in 23 (70%). Laparoscopic grade of disease among these 23 was mild in 10 (44%), moderate in 6 (26%), and severe in 7 (30%). Among the other 10 patients, the final diagnoses were viral enteritis (3 patients), ovulation (2 patients), pelvic adhesive disease (1 patient), degenerating uterine myoma (1 patient), endometriosis externa (1 patient), benign cystic teratoma of the ovary with rupture (1 patient), and ectopic pregnancy (1 patient). Epidemiologic data are as follows for patients with pelvic inflammatory disease by laparoscopy: age 23.4 ± 5.2 years; previous pelvic inflammatory disease in 10 patients (43%); history of any sexually transmitted disease in 17 (74%); multiple sex partners in the previous year in 12 (52%); sex partner treated for sexually transmitted disease in the previous month in 2 (9%); genital symptoms in a current sex partner in 2 (9%); history of intrauterine contraceptive device use in 6 (26%); oral, intrauterine contraceptive device, and no contraception in 6 (26%), 1 (4%), and 14 (61%), respectively; tubal sterilization in 2 (9%); and history of infertility and ectopic pregnancy in 11 (48%) and 1 (4%), respectively. We found no difference among patients without pelvic inflammatory disease for any of these parameters. Evaluation of symptoms of the current illness for those with pelvic inflammatory disease revealed a longest duration of any symptom of 6.3 ± 6.0 days, any bowel symptom in 19 (83%), any urinary symptom in 14 (61%), abnormal vaginal discharge in 9 (39%), abnormal uterine bleeding in 9 (39%), lateralization of pelvic pain in 12 (52%), fever in 14 (61%), chills in 17 (74%), onset during the follicular phase in 14 of 20 (70%), and total mean symptom score of 9.0 ± 2.9. Although there was no difference between the pelvic inflammatory disease and non–pelvic inflammatory disease groups for any of these symptoms, the frequencies of pain involving the upper abdomen were 11 (48%) and 1 (10%), respectively ($p < 0.05$). Table II shows a comparison among patients with and without pelvic inflammatory disease by laparoscopy of examination and laboratory data commonly used in the clinical diagnosis of pelvic inflammatory disease. In the group with pelvic inflammatory disease by laparoscopy, admission examination suggested an inflammatory pelvic mass in 8 patients, which was confirmed by laparoscopy in 5; inflammatory masses were not detected on examination in 2 patients.

Of the 23 patients with laparoscopy-documented pelvic inflammatory disease, 11 were randomized to treatment with cefamandole and clindamycin and 12 to doxycycline and clindamycin. One patient from the former group was unevaluable; she was doing well on the second day of therapy when she left the hospital. Severity of illness was equivalent in the two groups by clinical and laparoscopic parameters. Both regimens yielded 100% efficacy with no significant differences in days of therapy to response. No significant adverse drug reactions were recorded. Only five patients attended the visit 1 month after discharge, and all were doing well. All patients were contacted at 10 days after discharge, and no subjective relapses occurred in either group in spite of termination of antibiotic therapy at

Table III. Microbiologic findings from the cervix and fallopian tubes of 23 patients with laparoscopy-documented pelvic inflammatory disease

Isolate	*Clindamycin + doxycycline*		*Clindamycin + cefamandole*	
	Cervix (n = 11)*	*Tubal (n = 10)*	*Cervix (n = 12)*	*Tubal (n = 8)*
N. gonorrhoeae	6	5	3	1
C. trachomatis	2	0	4	1
Aerobes and anaerobes	—	0	—	3†

*Number of patients with cultures taken from site; only one of 23 patients had both gonococci and chlamydia on cultures from the endocervix (in doxycycline group), and no patient had both these organisms in the upper tract.

†Isolates from the three patients who were negative for gonococci and chlamydia: *Mycoplasma hominis*; *Escherichia coli*; anaerobic gram-positive rod, *Peptostreptococcus asaccharolyticus*, and *Bacteroides* sp.

hospital discharge. Our microbiologic findings are shown in Table III.

Combining the two treatment groups, days of therapy to response did not differ as a function of initial total sign score, temperature ≥38.0° C, white blood cell count ≥10,000/mm³, pelvic mass on physical examination, or old adhesions on laparoscopic examination. However, days to response for mild, moderate, and severe pelvic inflammatory disease by laparoscopic grading were 2.3 ± 0.5, 2.7 ± 1.5, and 3.9 ± 1.5, respectively ($p < 0.05$). Presenting temperature, white blood cell count, total symptom score, total sign score, and erythrocyte sedimentation rate showed no significant correlation with laparoscopic grade of disease. Sequential daily mean total sign scores during successful therapy were 15.9, 12.7, 8.6, 4.5, 2.8, 2.2, 2.1, 2.8, and 3.5; mean score on day of response was 4.4.

In Table IV each of the six variables assessed daily during therapy is examined for whether its requirement for response to therapy (as defined in Table I) had been achieved relative to the day the patient met composite criteria for response to therapy. On the day before qualification for therapeutic response, only 2 (9%) of the 22 patients had met the criterion for normalization of pelvic tenderness on bimanual examination, and in only 15 (68%) patients did this variable meet the criterion on the day of therapeutic response, when all five of the other variables had done so.

Comment

In the only study similar to ours, Sweet et al.[9] studied 29 women selected for inpatient therapy for pelvic inflammatory disease. The diagnosis was made by any four of (1) lower abdominal pain or (2) tenderness, (3) cervical motion tenderness, (4) adnexal tenderness, (5) fever, or (6) leukocytosis. Laparoscopy revealed the specificity of clinical diagnosis by their criteria to be 90%. Our study, like that of Jacobson and Westrom,[1] imposed no diagnostic criteria on physicians referring patients to the study. However, in spite of our application of criteria defining severe disease to these patients before enrollment, our specificity of clinical diagnosis (70%) was similar to that found by Jacobson and Westrom[1] (65%). We conclude that the diagnostic criteria for pelvic inflammatory disease employed by Sweet et al.[9] offer specificity superior to those used by individual clinicians in this and other existing studies and to our severe disease criteria; the false-negative diagnosis rate sustained by their criteria and the accuracy compared to existing guidelines[4] are not known. Standardization of diagnostic criteria for pelvic inflammatory disease seems desirable. Although our study was not designed to evaluate this issue, we do not wish to understate the importance of providing optimal medical therapy to all patients with pelvic inflammatory disease of any severity, particularly those who are young, nulliparous, or at risk for noncompliance, to minimize tubal injury and its sequelae.

In an analysis of patients studied by Jacobson and Westrom[1] who had a first episode, otherwise unclassified, of pelvic inflammatory disease, Hadgu et al.[10] identified the presence of purulent vaginal discharge and erythrocyte sedimentation rate ≥15 mm/hr as the strongest predictors of the presence of pelvic inflammatory disease. In patients having clinically severe pelvic inflammatory disease by our criteria, these two variables were not useful discriminators. Type II error may explain the failure of our study to confirm the value of certain historic features in diagnosis of pelvic inflammatory disease that others have shown, as reviewed by Eschenbach[11]—multiple sexual partners, genital symptoms in sexual partner, history of pelvic inflammatory disease, sexually transmitted disease, or ectopic pregnancy, and public funding. However, we believe that the practical clinical usefulness of these variables is truly limited by their high frequency in the subpopulation considered for a diagnosis of pelvic inflammatory disease, unwillingness of patients to divulge such information because of its negative connotations, or the fragmentary medical record often available when the patient is first seen. Our data do support the association between florid findings on physical exami-

Table IV. Status of individual outcome variables relative to satisfaction of composite criteria for therapeutic response* (n = 22 patients)

Outcome variable	*No. of patients for whom outcome variable met requirement for therapeutic response*		
	Day before composite response criteria were met	*Day composite response criteria were met*	*Day of discharge*
Cervical motion or adnexal tenderness	2	15	21
Abdominal findings	9	20	22
Symptoms	11	22	22
Temperature	14	20	22
White blood cell count	19	22	22
Pelvic mass	22	22	22

*See Table I and text for definition of variables and response criteria.

nation and documented pelvic inflammatory disease; lesser physical findings argue against it, regardless of other laboratory or historic data and even our clinical criteria for severe disease. Further, we found that the presence of bowel symptoms, lateralization of pelvic pain, and extension of pain to the upper abdomen do not argue against the diagnosis of pelvic inflammatory disease.

Rate of recovery, which may be considered a component in the definition of severity of disease, correlated among our patients only with laparoscopic grade of disease. Neither the presence of a pelvic mass by examination nor other data available without laparoscopy predicted rate of recovery, and none correlated with laparoscopic grade of disease. We take these data to support the accuracy and usefulness of the laparoscopic grading system. Because our clinical criteria for severe pelvic inflammatory disease were met by 10 (44%) of 23 patients with mild disease by laparoscopy, we cannot recommend the accuracy of these clinical criteria. Laparoscopy also provided an objective diagnosis for all 10 patients without pelvic inflammatory disease, who otherwise would have had continued illness or ineffective treatment until an accurate diagnosis was made later or not at all. However, we must caution that laparoscopy may yield false-negative results for pelvic inflammatory disease; exclusively endosalpingeal infection may occur without laparoscopically visible manifestations. A potential example of this phenomenon is our patient who had endocervical gonococcal infection and whose laparoscopic diagnosis was severe endometriosis.

Our outcome classification system, which also constitutes a management plan for inpatient therapy of pelvic inflammatory disease, is based on readily available clinical data. Its accuracy in defining adequate therapy is strongly supported by the absence of subjective evidence of relapsing disease in all of our patients. Although our criteria for therapeutic failure were not tested by this study population and would ethically be very difficult to test in any study, they represent the converse of the accurate response criteria and may be supported on that basis. Not only has this system been useful to us in subsequent therapeutic trials, but it is adaptable to general clinical use. We wish to emphasize that pelvic tenderness on bimanual examination (which can be performed from the bedside in <1 minute) is the most sensitive indicator of the need for continued treatment, as shown in Table IV. Because it is the one response variable that is abnormal at presentation in essentially all patients with pelvic inflammatory disease and that provides specific information about the site of infection, we strongly endorse its use. We find no data to support the concept that a brief, gentle daily pelvic examination could be detrimental to the patient's recovery. Our data also support the clinical applicability of the total sign score, which we adapted from Thompson et al.[12] A higher score significantly predicted the presence of pelvic inflammatory disease, and the mean sign score showed a linear decline during successful therapy until day 7, when only four patients remained.

Our two antibiotic combinations were designed to cover the bacterial spectrum encountered in pelvic inflammatory disease.[13] The high prevalence of *N. gonorrhoeae* in our area is reflected in the frequency of this organism in the lower and upper genital tract of patients in this study, 39% and 26%, respectively. Isolations of *C. trachomatis* from endocervix and oviduct were not as congruent (26% and 6%, respectively). Ten of the 18 tubal specimens (55%) grew a pathogen; our frequency of negative cultures is comparable to that in most reports on this subject. Both study drug regimens yielded 100% response rates by our criteria within a comparable interval, and there were no adverse drug reactions. Availability of our patients after discharge provided only for subjective assessment for relapsing infection, which did not occur with either regimen in spite of the absence of oral antibiotic therapy after discharge, a finding previously reported by Hager at al.[14]

for pelvic infections other than pelvic inflammatory disease. Nonetheless, we continue to recommend completion of 7 days' therapy for presumptive or documented chlamydial infection in this setting.

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Fetal maternal laryngeal papillomatosis in pregnancy: A case report

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Although genital condylomata worsen with pregnancy, we are unaware of any reports of fatal maternal laryngeal papillomatosis. A maternal death at 20 weeks' gestation of a patient with chronic laryngeal papillomatosis is described. Pregnant patients with a history of this disorder who have symptoms should be considered candidates for immediate evaluation. (Am J Obstet Gynecol 1992;166:524-5.)

Key words: Laryngeal papillomatosis, pregnancy, papillomavirus

Genital condylomata are being diagnosed more frequently in gynecologic and obstetric practices in many areas of the United States. The tendency of these lesions to proliferate in the genital area during pregnancy has long been noted. Infrequently laryngeal papillomatosis develops in children; it is acquired at delivery and is caused by the same papillomavirus that causes the genital lesions. We are unaware of any previous case reports in the medical literature of maternal complications arising from the presence of laryngeal papillomatosis in the mother. The suddenly occurring maternal death in this case underscores the potential seriousness of laryngeal papillomatosis in pregnancy.

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Case report

A 24-year-old woman, gravida 5, para 4, presented to our Obstetrical Clinic at 20 weeks' gestation. She was first diagnosed with laryngeal papillomatosis at age 2½,

with pathologic confirmation. Over the next 8 years she underwent numerous papillectomies by various physicians. The patient was untreated over the ensuing 15 years with apparent remission. Her only medical treatment from September 1985 to July 1990 was for gynecologic problems, spontaneous abortions, and delivery of infants without prenatal care. During this period she did not have respiratory distress, hoarseness, or audible respirations.

At the patient's first visit to the obstetric clinic during this pregnancy, she was noted to have audible respirations. She was not in respiratory distress. She did not keep the otolaryngology appointment that was scheduled at her first prenatal visit. At her next prenatal visit a second otolaryngology appointment was made, but the patient did not keep that appointment either. On her third visit she was noted to be hoarse with audible respirations but without respiratory distress. A third otolaryngology appointment was made for the patient. On the following day, the patient's family noted her to be in her usual state of health and without new respiratory symptoms. The next morning, she was found to be in acute, severe respiratory distress. On arrival at the hospital, she experienced a cardiopulmonary arrest, and intubation was difficult. She spontaneously aborted the next day and died on hospital day 10. Autopsy was refused. Histologic examination of the tissue recovered at the time of intubation revealed classic papillomatosis. A spot blot analysis indicated human papillomavirus type 6 or 11. A Southern blot confirmed type 11 virus.

Comment

This is apparently the first report of a maternal death from laryngeal papillomatosis during pregnancy. Although autopsy was refused, the diagnosis was histologically proved. The inciting event remains unknown and may have been a ball-valve effect or localized infection with edema. Whatever the immediate biologic mechanism, the certainty of laryngeal papillomatosis as the underlying cause requires obstetricians to be aware of the lethal potential of this disorder. Papillomavirus is becoming very widespread at the end of the 20th century; complications from maternal laryngeal papillomatosis in the future may not be as rare as they have been in the past. This patient had no obvious compromise to the immune system (including being human immunodeficiency virus negative), and the cause of reactivation of the disease at this time in her life is apparently attributable only to the pregnancy. Why this pregnancy and not prior ones would have caused such a problem is unclear.

The type 11 papillomavirus is one of the widely recognized genital condyloma types. Its finding is consistent with the patient's childhood disease and presumed acquisition at birth. Interestingly, human papillomavirus type 11 has generally and strongly been associated with nonaggressive disease.

The devastating effect of laryngeal papillomatosis has prompted many case reports of severely affected children. In 1871, MacKenzie[1] noted the frequent association of skin warts with laryngeal papilloma. Most current literature now suggests that approximately 60% of affected children have mothers with overt genital condyloma. With only one reported exception, all children with laryngeal papillomatosis have been delivered vaginally, thus suggesting that transmission is by direct contact rather than transplacentally.[2]

It is recognized that maternal condylomata frequently worsen with pregnancy. This heretofore unreported lethal complication of laryngeal papillomatosis suggests that pregnant women with even mild symptoms of laryngeal papillomatosis may benefit from aggressive otolaryngeal evaluation. Surgical intervention may be required during pregnancy.

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The function of elevated plasma fibronectin in preeclampsia

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Total plasma fibronectin is elevated in preeclampsia due to vascular injury release, increased production, or enzymatic degradation resulting in multimers. To examine the etiology of the fibronectin increase in preeclampsia, we quantified plasma fibronectin in nonpregnant women, pregnant women from 28 to 42 weeks' gestation, latent labor, and preeclampsia by both nephelometry and turbidimetry. Western blotting and gel electrophoresis were used to examine the structural integrity of the fibronectin molecule. In addition, functional assays explored the potential for dysfunctional fibronectin. Fibronectin was elevated in pregnant patients compared with nonpregnant patients and exhibited a further significant increase with preeclampsia. The increase was not a result of degradation to multimers but possibly to increased variants. Notably, fibronectin function, as defined by collagen binding, may be impaired during pregnancy and preeclampsia. It appears that the clinical pathophysiology of preeclampsia may be related to dysfunctional fibronectin measured by collagen binding. (AM J OBSTET GYNECOL 1992;166:526-31.)

Key words: Fibronectin, preeclampsia, collagen binding

Fibronectins are large, usually dimeric, glycoproteins (molecular weight approximately 450,000 D) that are found both in a soluble form in plasma (plasma fibronectin) and in a larger, nonsoluble form in the extracellular matrix (cellular fibronectin).[1-4] Plasma fibronectin is synthesized primarily by hepatocytes[5,6] and is involved in opsonic activities with the reticuloendothelial system and in clot stabilization. Cellular fibronectin, synthesized by fibroblasts, endothelial cells, and astroglial cells among others, is involved in cell adhesion, migration, growth and differentiation.[1-3,7,8]

The various different fibronectins are derived from a single fibronectin gene and are composed of multiple domains. The exons making up the fibronectin gene correspond directly to functional domains of the protein. The sequence and presence or absence of the various domains accounts for the differing functional activities of the different fibronectins.[1-4,9] These activities include binding to fibrin, collagen, cells, and heparin. There are at least 12 versions of fibronectin,[3] although the fibronectin gene itself does not vary. These different variants are caused by alternative splicing of the messenger ribonucleic acid, which is subsequently translated into the fibronectin protein. Different cell types (hepatic, fibroblast, and endothelial) may synthesize different fibronectin variants. One of these variants is a soluble, plasma fibronectin containing an extra type III domain (ED1+)[10] found predominantly in large vessel endothelial cells[11] and in very small quantities in platelets.[10] The plasma fibronectin synthesized by hepatocytes lacks the ED1+ domain. Although the function of this variant is unclear, it has been associated with endothelial cell damage.[12] The association of elevated total plasma fibronectin and ED1+ fibronectin both in pregnancy and in gestational hypertension and preeclampsia have been documented in previous studies with a variety of methods (turbidimetry, nephelometry, quantitative electroimmunoassay, dual electrosorbent, enzyme-linked immunoassay [ELISA] method, Laurell immunoelectrophoresis).[13-22] The etiology of the elevation in plasma fibronectin in preeclampsia is uncertain. Although it may be related to vascular injury (elevated ED1+) characteristic of preeclampsia, the majority of plasma fibronectin is produced in the liver. Thus the etiology of the total fibronectin increase in preeclampsia remains uncertain, but there are several possibilities, which include (1) amniotic fluid leakage into the maternal circulation, (2) increased synthesis or release of fibronectin variants other than (but including) ED1+, (3) increased production of hepatic fibronectin (acute phase reactant), (4) endothelial cell damage (leakage), (5) increased proteolytic enzyme degradation of fibronectin, (6) increased platelet production of fibronectin, (7) increased tissue release of fibronectin secondary to vasospasm and hypertension, or (8) decreased renal clearance of fibronectin.

The purpose of this study is to investigate whether elevated plasma fibronectin results from monomers or multiple fragments and whether fibronectin is dys-

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functional in this disease. We examine quantitative methods of fibronectin measurement (turbidimetry and nephelometry) as related to pregnancy, labor, and preeclampsia. We investigated the possibility that increases in fibronectin in pregnancy and preeclampsia are due to a structural change or cleavage to multimeric forms (by Western blotting and native gel electrophoresis) in the fibronectin molecule. Finally, we investigate functional assays of fibronectin (ELISA, Western blotting) to determine if the elevations of fibronectin in pregnancy and preeclampsia are caused by dysfunctional fibronectin.

Material and methods

We studied 82 patients divided into four groups: nonpregnant controls (10), pregnant control patients (23) at various nonlaboring gestational ages, pregnant patients (29) without preeclampsia in latent labor (cervix dilated <4 cm) at ≥36 weeks gestation, and patients (40) in labor with mild or severe preeclampsia at various gestational ages.[23] The pregnant controls and preeclamptic patients were subdivided into the following gestational age groups: 26 to 34 weeks, 35 to 38 weeks, and 39 to 42 weeks. We correlated fibronectin quantitation and function with gestational age to determine whether fibronectin was useful as a marker in the early detection of preeclampsia. Because most of the preeclampsia patients were only detected at delivery, we questioned the effect of labor on fibronectin We had no laboring patients before 36 weeks; therefore, all laboring controls were grouped in a category >36 weeks.

All patients and controls signed an informed consent form approved by the Human Subject Review Board of the Research and Educational Institute of Harbor–University of California, Los Angeles Medical Center. Blood samples were obtained by venipuncture and collected into citrated tubes. After centrifugation at 3000 rev/min for 10 minutes, plasma was frozen in multiple aliquots at −85° C.

Measurement of plasma fibronectin. Quantation of fibronectin by nephelometry and turbidimetry was performed as previously described by Brubaker et al.[24] We used undiluted rabbit anti-human fibronectin obtained from Biomedical Technologies, Inc. (Cambridge, Mass.) for the nephelometric method. The fibronectin standards, 1000 μg/ml (Biomedical Technologies, Inc., Cambridge, Mass., and Boehringer-Mannheim, Indianapolis, Ind.) was prepared at various dilutions to create a curve within the instrumentation. Controls of low (100 to 150 μg/ml), medium (300 to 500 μg/ml), and high (800 to 1000 μg/ml) were used between runs and for comparison with turbidimetric analysis.

The turbidimetric analysis was performed with the Boehringer-Mannheim Fibronectin (Opsonic Protein) assay kit. The suggested manufacturer's procedures were followed with a Gilfore Stasar III spectrophotometer.

The functional assay used in this study was a collagen-binding method with alkaline phosphatase–labeled fibronectin in a competitive inhibition assay. A modification of the method of Doran et al.[25] was used with soluble collagen, type X from human placenta (Type III, Sigma Chemical Co., St. Louis. Mo.) in the microtiter plates in place of gelatin. Several collagen types were tested in the modified Doran method; however, type X optimally bound fibronectin.

Fibronectin (native) protein electrophoresis was performed with 3% stacking polyacrylamide gels according to the method of Laemmli.[26] Fibronectin transfer by electrophoresis to nitrocellulose (Western blot) was similar to that of Towbin et al.[27] A fibronectin control was intentionally split into monomers with 2-mercaptoethanol at 37° C. Six antibodies were used in the immunoblotting method: polyclonal anti-human fibronectin, monoclonal antibodies to human fibronectin, monoclonal antibodies to human amniotic fibronectin, and monoclonal antibodies to fibronectin molecular domains for cell, heparin, and gelatin (collagen) attachment. All antibodies were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.).

Statistics. First- and second-order polynomial equations were used to describe the relationship of fibronectin values versus gestational age. Differences between the nonpregnant controls and pregnant controls, pregnant controls and pregnant patients in latent labor, and the three preeclamptic groups versus pregnant control groups were evaluated by unpaired t tests for each of the fibronectin assays. A Bonferroni correction was used for multiple comparisons in the three groups, with a $p \geq 0.0167$ considered significant. All results are expressed as mean ± SEM.

Results

The nonpregnant group consisted of 10 patients, seven nulliparous and three multiparous, mean age 35.3 ± 7.5 years. There were 23 pregnant control patients (nine nulliparous and 14 multiparous, mean age 24.2 ± 5.7 years). The mean fibronectin level was significantly greater in the pregnant versus nonpregnant patients as determined by nephelometry (433 ± 27 vs 252 ± 29 μg/ml; $p < 0.005$). There were no significant differences in fibronectin levels with turbidimetry (243 ± 20 vs 228 ± 15 μg/ml). With collagen binding, as determined with the ELISA method, the pregnant patients had significantly lower fibronectin values than nonpregnant controls (314 ± 51 vs 623 ± 35 μg/ml; $p < 0.005$).

The nonpreeclamptic patients in latent labor at ≥36 weeks' gestation consisted of nine patients (three nul-

Table I. Mean ± SEM fibronectin as determined by nephelometry, turbidimetry, and ELISA in preeclamptic and control pregnant patients at 26 to 34, 35 to 38, and 39 to 42 weeks' gestation

	26-34 weeks		*35-38 weeks*		*39-42 weeks*	
	Preeclamptic (n = 6)	*Control (n = 11)*	*Preeclamptic (n = 18)*	*Control (n = 6)*	*Preeclamptic (n = 16)*	*Control (n = 6)*
Nephelometry (μg/ml)	823 ± 39*	460 ± 42	873 ± 95*	338 ± 47	1064 ± 98*	478 ± 39
Turbidimetry (μg/ml)	430 ± 70*	229 ± 28	769 ± 128*	249 ± 52	647 ± 74*	263 ± 32
ELISA (μg/ml)	415 ± 90*	181 ± 42	450 ± 47	258 ± 47	515 ± 52	615 ± 97

*$p < 0.0167$ vs control.

liparous and six multiparous, mean age 22.4 ± 5.8 years). Eleven pregnant patients (five nulliparous and six multiparous, mean age 24.5 ± 6.4 years) not in labor and without preeclampsia at ≥36 weeks were used as controls to examine the effect of labor. Nonlaboring and laboring patients had similar fibronectin values by nephelometry (411 ± 39 vs 354 ± 55 μg/ml) and turbidimetry (264 ± 30 vs 268 ± 16 μg/ml). However, laboring patients demonstrated significantly lower fibronectin values by ELISA as compared with nonlaboring patients (273 ± 33 vs 458 ± 78, $p < 0.05$).

The best correlation of plasma fibronectin levels with gestational age was obtained with a second-order polynomial equation. For turbidimetry the equation is fibronectin = $2496.813 - 135.92\ (GA)^2 + 2.009$, $r = 0.46$, where GA is gestational age. For ELISA the equation was fibronectin = $4204.604 - 270.087\ (GA)^2 + 4.474$, $r = 0.75$. There was not a good correlation with nephelometry ($r = 0.05$).

There were a total of 40 preeclamptic patients (34 nulliparous and six multiparous, mean age 21.8 ± 4.6 years). When compared with control pregnant patients, preeclamptics had significantly higher levels of fibronectin with nephelometry and turbidimetry at all gestational ages (Table I). Statistically significant increases in collagen determined fibronectin was demonstrated only at 26 to 34 weeks' gestation.

All patient and control samples were immunoblotted (Western blot) to observe disulfide split into monomers or proteolytic cleavage into multimers with a polyclonal anti-human fibronectin. Sample 2 (Fig. 1) represents intentional disulfide splitting of the fibronectin molecule into monomers (second band) with a molecular weight of 220,000 D, whereas the first band represents the intact 440,000 D fibronectin molecule. In severe preeclampsia (sample 1), mild preeclampsia (sample 3), and normal pregnant control; (sample 4), the fibronectin molecule was intact for the polyclonal antibody and the monoclonal antibodies to gelatin (collagen-binding domain), heparin (aminoglycan-binding domain), and cell attachment domain on the fibronectin molecule. Fig. 1 is representative of all the patients' results.

Comment

Plasma fibronectin as determined by various methods is increased in normal pregnancy as compared with the nonpregnant state[17, 28, 29] and further increased in preeclampsia and gestational hypertension.[13-22] In the present study, we confirmed an increase in total plasma fibronectin by nephelometry, although not by turbidimetry. Studies by Thurneau et al.[18] with nephelometry, Eriksen et al.[17] with quantitative electroimmunoassay, and Hess et al.[28] and Brady et al.[29] with turbidimetry demonstrated increased fibronectin in the third trimester of normal pregnancy as compared with the nonpregnant state. Perhaps the difference in our results with nephelometry and turbidimetry is due to the increased sensitivity of nephelometry in detecting fibronectin variants in plasma.

There was no correlation between gestational age and fibronectin values with the nephelometry method. Turbidimetry showed values similar to those of nonpregnant controls throughout the third trimester, although these values tended to increase before 28 weeks and after 40 weeks ($r = 0.46$). Studies by Saleh et al.[19] (nephelometry), Ballegeer et al.[20] (immunoelectrophoresis), and Lockwood and Peters[21] (by dual ELISA method) with total plasma fibronectin did not show a rise in fibronectin values with advancing gestational age, although Hess et al.[28] (by turbidimetry) showed an increase in plasma fibronectin in pregnancy only in the third trimester. The ED1+ fraction did increase with gestational age in Lockwood and Peters' study.[21]

The results of the ELISA assay for collagen binding as a single assay can be interpreted as a measure of fibronectin dysfunction or the presence of a fibronectin variant. If the quantity is decreased in this assay, the fibronectin is dysfunctional in its ability to bind collagen (most likely interpretation) or there is a decrease in a collagen-binding variant. An elevation in this assay indicates a rise in a variant that is collagenophilic (most likely) or there is increased functional binding. The ELISA values as a single test were highly correlated with gestational age. Although low throughout the third trimester, the levels approached values of nonpregnant women near term. The decrease in ELISA

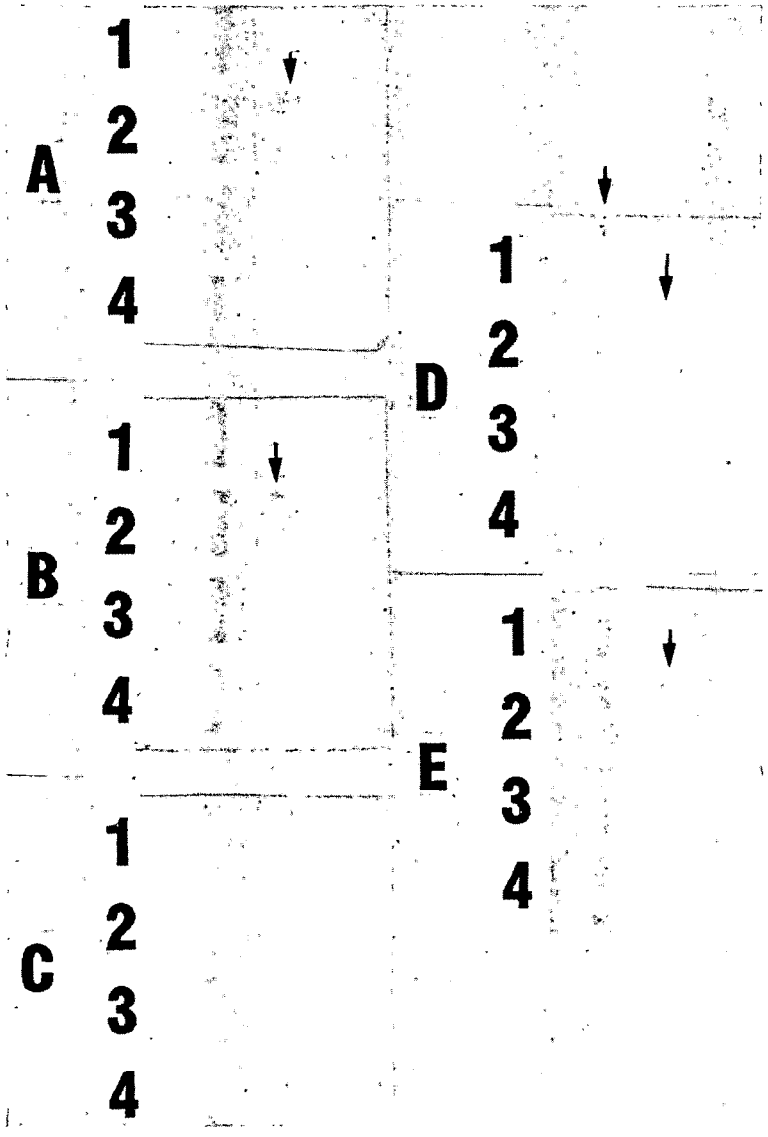


Fig. 1. Immunoblotting of samples from patient with severe preeclampsia *(lane 1)*, from intentionally split fibronectin *(lane 2)* with the monomer band below *arrow*, from patient with mild preeclampsia *(lane 3)*, and from normal pregnant control *(lane 4)*. The antibodies used to detect the fibronectin bands are **A,** monoclonal antibody to amniotic fluid fibronectin; **B,** monoclonal antibody to the cell-binding domain of fibronectin; **C,** monoclonal antibody to the collagen-binding domain of fibronectin; **D,** polyclonal antibody to fibronectin; and **E,** monoclonal antibody to heparin-binding domain of fibronectin.

values in pregnant patients possibly indicates that fibronectin may be dysfunctional for collagen binding in pregnancy. Labor did not affect fibronectin values as determined by nephelometry and turbidimetry, confirming the findings of Saleh et al.[18] and Brady et al.[29] The decreased ELISA values with labor may reflect further dysfunction of the fibronectin molecule. We have no definitive interpretation of the ELISA, but several biologic explanations are prudent. The decreased collagen binding in healthy pregnant women (gravid controls) in comparison with nongravid controls may be due to a healthy pregnant woman's response to a developing fetus, which is similar to the fibronectin response found in tumorigenesis.[4] This may represent dysfunctional fibronectin that becomes functional at term (third trimester levels are equivalent to nongravid levels) due to full development of the fetus. Another explanation for the increase in collagen binding near term may be a factor elaborated by the placenta, which is responsible for the decreased binding earlier in pregnancy, and with placental aging the production of this factor may diminish. The decrease found during de-

livery may represent lesser biologic collagen binding due to the release of the fetus at delivery. However, our ELISA assay must not be interpreted as a single assay but in conjunction with the quantitative results. The lack of increase in collagen binding by the ELISA assay in preeclampsia along with the increase in total fibronectin indicates either the secretion of a functionally altered fibronectin variant(s) or a circulating factor that alters collagen-binding.

Stubbs et al.[13] originally postulated that the increased plasma fibronectin is a result of release from damaged endothelial cells or a result of increased endothelial cell synthesis. Serum from preeclamptic women was found to be cytotoxic to cultured endothelial cells,[30] suggesting release of fibronectin from injured endothelium. The association of variant ED1+ fibronectin with endothelial cell injury and the discovery of elevated ED1+ fibronectin in preeclampsia[21] further substantiates the endothelial cell injury hypothesis. However, the endothelial injury theory is not fully substantiated and requires further study. Our nephelometry method suggests variants exist in normal pregnancy and in preeclampsia, because total fibronectin values are higher with nephelometry than turbidimetry. This finding is especially obvious when comparing nephelometry and turbidimetry in the experimental subjects (normal pregnancy and preeclampsia) with nonpregnant controls, where there is no significant quantitative difference between the methods in the nonpregnant women but there are differences in the experimental subjects. This finding results from the increased sensitivity of nephelometry. Increased sensitivity is due to amplification of a small signal on the black background in nephelometry compared with detecting an intermediate decrease in a large signal in turbidimetry.

No previous study has evaluated proteolytic cleavage of fibronectin into multimers or fragments. This also is a potential explanation of the elevated fibronectin in preeclampsia, especially because all quantitative methods are immunoassays. The results of immunoblotting have demonstrated that the 440,000 D fibronectin molecule is totally intact in preeclampsia. Using the monoclonal antibody to amniotic fluid fibronectin, the bands appeared wider and stronger in pregnant women and accentuated in severe preeclampsia. A quantitative assay will further delineate this observation.

The explanation for elevated fibronectin in preeclampsia still remains an enigma. In summary, pregnancy is associated with an increase in total fibronectin by nephelometry with a further increase by both nephelometry and turbidimetry in preeclamspia. The decrease in fibronectin function (ELISA-measured collagen binding) in normal pregnant patients and the lack of increase in patients with preeclampsia (>34 weeks) suggests either the secretion of functionally altered fibronectin variant(s) or a circulating factor of pregnancy that alters fibronectin function.

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The Zavanelli maneuver applied to locked twins

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The Zavanelli maneuver was applied to locked twins. After the first infant was returned into the vagina, an emergency cesarean section was performed. After a not-too-good start, both children did well. The Zavanelli maneuver is presented as a method to reverse the potentially catastrophic situation of locked twins. (AM J OBSTET GYNECOL 1992;166:532.)

Key words: Zavanelli maneuver, twins, locked twins

The Zavanelli maneuver is indeed a revolutionary concept for handling obstructed delivery.[1] The results have indicated that return into the vagina of a partially delivered infant and subsequent cesarean section might have a better neonatal outcome than forced completion of vaginal delivery. This case report describes the successful application of the Zavanelli maneuver to locked twins.

Case report

A 33-year-old secundigravid woman was expecting twins. The diagnosis was made in early pregnancy by ultrasonography, which demonstrated two separate gestational sacks, both with living fetuses. At 36 weeks 6 days' gestation she was admitted to the hospital with spontaneous rupture of the membranes of the first fetus. Except for a slight rise in diastolic blood pressure, the course of pregnancy had been uneventful. Uterine fundal height and ultrasonographic measurements showed satisfactory fetal development.

While labor progressed, the positions of both fetuses were determined by ultrasonography. The first fetus was in a breech presentation, and the second one was in a position between transverse and head down (head at the level of the maternal umbilicus). Because of the position of the second child, the risk for interlocking was estimated to be small. Vaginal delivery was accepted. Frequent ultrasonographic localization of the head of the second child was performed. At 18 minutes after active pushing had begun, the breech of the first child crowned at the vulvar ring. At this time we could not localize the head of the second child. With the next contraction the first child was delivered to the lower edges of the shoulder blades. Further delivery was impossible. On vaginal examination interlocking was recognized. We returned the partially delivered first infant into the vagina (moderate force was required). Emergency cesarean section was performed 10 minutes later, and two female infants were delivered. The Apgar scores of the first child after 1 and 5 minutes were 1 and 4; those of the second child were 2 and 4. Birth weights were 2580 and 2000 gm, respectively. Both children were cared for on the neonatal ward and both did well. Twelve days after cesarean section the two girls left the hospital with their mother.

Comment

Interlocking is a rare complication of twin delivery with a high mortality, especially for the first child. Pushing up the head of the second child or performing a cesarean section may be possible in the early stage. As interlocking proceeds, e.g., after breech extraction is attempted, few possibilities remain. In the case of a large pelvis or small fetal mass the Kimball-Rand procedure can be of help. If not, sacrificing one child to save the other might be the only remaining option.

The current case is the second report in the literature of the application of the Zavanelli maneuver to locked twins. Iffy et al.[2] were confronted with interlocking during preparation for elective cesarean section. After interlocking was recognized, it took only 3 minutes until abdominal delivery. The first child was born by traction to the head combined with pushing to the breech. In our case the child was completely returned into the vagina before an emergency cesarean section was performed about 10 minutes later. In our opinion the Zavanelli maneuver can be of help to reverse the potentially catastrophic situation of locked twins.

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Radical hysterectomy for treatment of cervical cancer: A prospective study of two methods of closed-suction drainage

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Two closed-suction drainage methods were prospectively compared in 96 patients after radical hysterectomy with pelvic lymphadenectomy in stage IB cervical cancer. In group 1 (n = 49) two pelvic sidewall drains and a vaginal drain were used, and in group 2 (n = 47) only the vaginal drain was used. The groups were similar for mean age, preoperative weight, hemoglobin and serum albumin level, operating time, operative blood loss, and blood transfusions. The febrile morbidity rates and the operative site infection rates were similar in the two groups. Ninety vaginal drains were removed by day 3. By day 6 55% of patients in group 1 had at least one sidewall drain, with a mean drainage of 150 ml/day. The median postoperative stay was similar in both groups. A pelvic lymphocyst developed in one patient in each group. The single vaginal closed-suction drain is safe, efficient, more acceptable to patients, and more cost-effective. (Am J Obstet Gynecol 1992;166:533-7.)

Key words: Radical hysterectomy, cervical cancer, closed-suction drains

The early stages of invasive carcinoma of the cervix are treated by either radical surgery or radiotherapy.[1] Although not universal, traditional surgical practice after radical hysterectomy and pelvic lymphadenectomy has been to reapproximate the pelvic peritoneum and to drain the pelvis. Pelvic drainage, recommended because of increased pelvic fluid formation, was used to prevent pelvic hematomas and reduce postoperative pelvic infection, pelvic lymphocyst formation, and ureteral complications.[2, 3]

The benefit of prophylactic antibiotics in reducing infectious morbidity, however, seemed unclear.[4, 5] Furthermore, the value of and the method of prophylactic drainage have remained controversial, and inappropriate drainage has been associated with significant morbidity.[6-8] Currently, closed-suction drainage systems with low-pressure vacuum reservoirs are advocated.[6-8] In this division two closed-suction pelvic sidewall drains and a closed-suction vaginal drain have been used after radical hysterectomy and pelvic lymphadenectomy,[5] but this method may not confer any advantage over a single, closed-suction vaginal drain. It was hypothesized that one vaginal suction drain would not reduce pelvic fluid drainage, duration of use, and rate of pelvic soft tissue infection or increase patient acceptability compared to one vaginal and two sidewall drains. Because pelvic drainage methods after radical hysterectomy and pelvic lymphadenectomy have received little specific attention in recent gynecologic literature, a prospective study was undertaken to test this hypothesis.

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Material and methods

Between July 1987 and November 1990, 96 patients who underwent a radical hysterectomy and bilateral pelvic lymphadenectomy for treatment of stage IB (International Federation of Gynecology and Obstetrics) invasive carcinoma of the cervix were entered in the study. All operations were performed by members of the Division of Gynecologic Oncology. No patient received radiotherapy before surgery. The preoperative procedure for all patients included a mechanical bowel preparation (without antibiotics). The operative procedure was standard with a vertical midline abdominal incision followed by a Rutledge type III operation[9] with a selective paraaortic lymphadenectomy. The pelvic peritoneum was not reapproximated. In younger patients the ovaries were conserved and transposed. The abdominal wall was closed with the continuous mass-closure technique[10] with either No. 2 polyglactin suture (Vicryl; Ethicon, Somerville, N.J.), or No. 1 polyglyconate (Maxon; Davis and Geck, Danbury, Conn.). The method of bladder drainage was transurethral with an 18F Foley catheter left in situ for approximately 6 weeks, during which time patients received prophylactic nitrofurantoin, 100 mg daily.

When three drains were used, one drain (T tube) was brought out through the vagina, and each of the other drains was placed at the pelvic sidewall and brought

Table I. Patient characteristics

	Group 1 (n = 49)		*Group 2 (n = 47)*		
	Range	*Mean*	*Range*	*Mean*	*Significance*
Age (yr)	22-70	44.8	27-77	47.2	NS
Preoperative weight (lb)	105-263	150.6	95-308	155.9	NS
Preoperative hemoglobin (gm/dl)	10.4-16.4	12.8	10.2-15.1	13.0	NS
Preoperative serum albumin (gm/dl)	1.6-4.9	4.1	3.5-4.8	4.2	NS

NS, Not significant.

Table II. Operative and postoperative details

	Group 1 (n = 49)	*Group 2 (n = 47)*	*Significance*
Operating time (hr, range and mean)	2.75-5.8 (4.3)	3.1-5.8 (4.2)	NS
Blood loss (ml, range and mean)	400-2400 (923)	350-2400 (942)	NS
Blood loss >1000 ml (No.)	15	17	NS
Intraoperative transfusion (No.)	12	7	NS
Total cases transfused (No.)	19	11	NS
Paraaortic lymphadenectomy (No.)	24	21	NS
Metastatic nodal disease (No.)	4	11	NS
Postoperative stay (days, range and mean)	6-7 (7.4)	6-16 (6.4)	NS
Day 3 postoperative hemoglobin (gm/dl, range and mean)	7.2-13.0 (10.2)	8.0-11.3 (9.6)	NS
Day 3 postoperative serum albumin (gm/dl, range and mean)	2.1-3.4 (3.0)	2.2-3.9 (3.0)	NS

NS, Not significant.

out extraperitoneally through the anterior abdominal wall.[5] The rectosigmoid colon was mobilized into the pelvis to reduce the pelvic depth and prevent small bowel from adhering in the pelvis. All three drains were attached to low suction. The vaginal drain was a round 19F, 6.3 mm outer diameter, Jackson Pratt T-tube hysterectomy drain (Heyer-Schulte, American V. Mueller, Chicago). The pelvic sidewall drains were flat (Jackson-Pratt Hubless 10 mm flat silicone drains, 20 cm × 10 mm × 4 mm). Each drain was attached to a soft, pliable, closed-suction 100 ml reservoir that has an antireflux valve (Meyer-Schulte, American V. Mueller, Chicago). Patients operated on between July 1987 and June 1989 had the vaginal T tube and two sidewall drains, and those patients operated on after June 1989 had the vaginal T-tube drain only.

All patients received prophylactic antibiotics 1 hour before surgery and then 6 hours and 12 hours after surgery. Antithrombotic measures were prophylactic heparin, 5000 U subcutaneously, before surgery and every 12 hours thereafter, or Venodyne compression boots (Advanced Instruments, Inc., Needham Heights, Mass.) until the patient was fully ambulatory. More recently only the latter method has been used except in patients with a history of thromboembolism. The volume of fluid from each drain was measured daily. The drains were removed if the volume collected in 24 hours was <30 ml. Postoperative febrile morbidity was strictly defined as two consecutive oral temperatures of >38° C (100.4° F) 4 hours apart, excluding the first 24 hours after surgery. Two consecutive temperatures >38° C represented failure of antibiotic prophylaxis, and patients were treated with other antibiotics. The causes of febrile morbidity were defined as operative site infection (pelvis), non-operative-site infection (for example, chest infection or urinary infection), and source unknown. In the absence of any known non-operative-site infection and in the presence of persistent febrile morbidity the source was presumed to be a pelvic soft tissue infection.[4, 5, 11-13] Results in the two groups were compared by χ^2 analysis for dichotomous measurements and the Student t test for continuous measurements. The level of significance was $p < 0.05$.

Results

There were 49 patients with a vaginal drain and two pelvic sidewall drains (group 1) and 47 with the vaginal drain only (group 2). Aside from the chronologic allocation of patients to the two groups there was no identifiable selection bias. The two groups had similar demographic features (Table I). More patients ($p > 0.05$) in group 2 had metastatic nodal disease (Table II). Intraoperative blood loss was similar in patients receiving heparin and compression boots.

The drainage volumes for the first 3 postoperative days are shown in Table III. The vaginal T tube was

Table III. Mean pelvic drainage

Postoperative day	Group 1			Group 2; vaginal drain (ml)	Significance
	Vaginal drain (ml)	Sidewall drains (ml)	Total (ml)		
1	52	187	239	80	S
2	32	215	247	43	S
3	18	179	197	18	S

S, Significant.

Table IV. Mean daily volume from pelvic sidewall drains in group 1

	Postoperative day								
	1	2	3	4	5	6	7	8	9
No. of patients with one or both sidewall drains	49	49	49	49	48	37	27	13	9
Volume drained (ml)	187	215	179	159	160	147	138	141	79

Table V. Febrile morbidity

	Group 1 (n = 49)		Group 2 (n = 47)		Significance
	No.	%	No.	%	
Total cases	24	48.9	22	46.8	NS
Surgical site	13	26.5	9	19.1	NS
Nonsurgical site	11	22.4	13	27.7	NS

NS, Not significant.

removed on the third postoperative day, except in three patients in each group in whom it was removed on day 4. No patient complained of vaginal drainage after the drain had been removed. In group 1 most of the drainage was from the pelvic sidewall drains and remained high, so that by day 7 27 patients (55.1%) had at least one sidewall drain (Table IV). The volume of pelvic drainage was not related to patient weight, operative time, blood loss, or method of thrombotic prophylaxis. The prolonged continued use of the sidewall drains was a source of patient inconvenience and discomfort.

The incidence and causes of febrile morbidity in the two groups are comparable (Table V). No patient had serious surgical site infection. Forty-six of the 96 patients (47.9%) had febrile morbidity. Twenty-two (22.9%) had operative site infection, but their drainage volumes were similar to those of patients who had no febrile morbidity. Fifteen (15.6%) patients had metastatic nodal disease. Ten of these had postoperative febrile morbidity, compared with 34 of 81 patients without nodal disease ($p < 0.05$). Half of these patients with febrile morbidity, regardless of nodal status, had surgical site infection. In group 1 the mean vaginal drainage volumes were similar in those with and without nodal disease. Although there was an impression of greater sidewall drainage in the first three postoperative days in node-positive compared with node-negative patients, meaningful statistical analyses were not applicable because only four patients in group 2 had positive nodes. In group 2 the mean daily vaginal drainage volumes were similar in node-positive and node-negative patients. The mean total daily drainage volumes for the first three postoperative days were similar in the node-negative (n = 81) and node-positive patients (n = 15).

The median postoperative stay was 7 days (range 6 to 15) in group 1 and 6 days (range 6 to 16) in group 2. There have been two pelvic lymphocysts, one in each group, both asymptomatic and detected clinically at 2 and 12 weeks after surgery. Both patients had metastatic pelvic nodal disease. There were no pelvic abscesses or ureterovaginal fistulas.

Comment

The results indicate that one vaginal closed-suction drain was a safe, effective alternative to two pelvic sidewall drains and a vaginal drain after radical hysterectomy and bilateral pelvic lymphadenectomy in stage IB cervical cancer. Furthermore, patients with pelvic sidewall drains continued to have significant pelvic drainage after the vaginal drain had been removed. How-

ever, the pelvic soft tissue infection rates in the two groups were comparable.

Variables such as the type of drain (closed or open, suction or nonsuction), the number of drains used, and whether the pelvic peritoneum was approximated have often not been considered in reports on morbidity from radical hysterectomy where, in addition, the surgical technique has differed.[3-4, 11-13] This makes it difficult to determine what factors are important in morbidity. In this study the surgical technique was standardized. We used closed-suction, low-pressure drainage systems, which have been advocated in other studies and which are associated with less ascending infection and less trauma to healing tissues, including the peritoneum.[6, 14-16]

One aim of pelvic drainage is to reduce lymphocyst formation, the incidence of which, after radical surgery in cases of cervical cancer, varies,[17-19] with reports of almost 50%.[19] Because not all of the lymphatics can be removed or ligated at a lymphadenectomy and lymph fluid is produced continually, a low incidence of lymphocyst formation might be expected, regardless of the method of drainage. In contrast, pelvic drains have been reported to increase the incidence of lymphocyst formation.[17, 18] Most pelvic lymphocysts are detected in the early postoperative period,[17-19] as in the two cases recorded in this study. It had been suggested that nonclosure of the pelvic peritoneum would reduce lymphocyst formation, but this was not confirmed in early reports.[18, 19] It has also been suggested that one reason lymphocysts do not follow all lymphadenectomies is that lymph fluid escapes into the peritoneal cavity, where it is absorbed.[19] This is of interest because a treatment of pelvic lymphocysts has been marsupialization.

The peritoneum has highly efficient reparative[20, 21] and absorptive[22] capacities, and closure of the pelvic peritoneum, while arguably unnecessary, is also time-consuming and may be associated with trauma to adjacent structures, such as the ureter.[15, 20] Damaged peritoneum heals normally without adhesions unless infection, ischemia, or foreign bodies are present.[20, 21] A pelvic drain could, if left in situ for some days, impair the reparative and absorptive capacities of the peritoneum and so contribute to the very problems its use was intended to prevent.[15] This suggests that drains at peritoneal surfaces should be removed early or not be used at all.

The two cases of pelvic lymphocysts were in patients with nodal metastases in whom pelvic drainage and lymphocyst formation[18] is reported to be higher. We found no significant difference in the mean daily drainages in node-positive compared with node-negative patients, either within each group or in the study population as a whole. We did observe, however, that patients in group 1 continued to have high drainage after the vaginal drain had been removed. This suggests that either these low-pressure pelvic side wall drains contributed to the continued pelvic drainage[17] (by maintaining patency of lymph vessels and reducing the absorptive capacity of the peritoneal surfaces) or the drainage was absorbed effectively in group 2 by the peritoneal surfaces.[22]

Pelvic drainage fluid contains electrolytes and protein, and continued fluid loss is not in the patient's best interest.[23, 24] Many patients with cervical cancer are nutritionally depleted before treatment and usually more so after treatment.[25] Group 1 patients had high total drainage volume (≥1 L compared with <200 ml in group 2). High pelvic drainage has been previously reported with sidewall drains.[3, 11, 24] The high pelvic drainage in group 1 continued to postoperative day 6 in more than half the patients. Other studies with different drainage methods and varying surgical techniques reported high loss on the first day or up to the third[23] and fifth days.[24] A previous study reported no difference in total pelvic drainage in patients with two and one drain,[24] but different surgical procedures had been performed in that study. Our results clearly show a higher total volume of drainage in patients with three drains. Prolonged use of drains also caused inconvenience and discomfort for the patients and was associated with a trend toward increased hospital stay.

Febrile morbidity is common after radical hysterectomy but serious postoperative infections are not and no serious operative site infections occurred. The febrile morbidity rates due to surgical site infection in the two study groups were similar and comparable to those in other reports.[3-5, 11-13] In a larger number of patients a significant difference between the two groups might have been demonstrated. Patients who had surgical site infection ($n = 22$) had mean daily total drainage volumes similar to those of patients without febrile morbidity ($n = 50$). In fact, a sudden drop in drainage volume was viewed as possibly representing blockage of a drain, and such drains were carefully stripped. Patients with metastatic nodal disease were more likely to have surgical site infection than those without nodal disease. This association was not reported in the studies reviewed.[3-5, 11-13] Febrile morbidity was not associated with low or high pelvic drainage, as reported previously.[24]

To our knowledge, this is the first prospective study to specifically compare the value of different drainage methods after radical hysterectomy and pelvic lymphadenectomy for stage IB cervical cancer. Patients with pelvic side wall drains had prolonged drainage. This was associated with increased patient discomfort, inconvenience, and expense (about $100 for drains and cost of increased hospitalization). On the basis of these facts we recommend that, in addition to not reapprox-

imating the pelvic peritoneum, a single closed-suction, low-pressure vaginal T-tube drain be used. This is now our practice for all patients undergoing radical hysterectomy and pelvic lymphadenectomy.

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Arterial pressure response to maximal isometric exercise in pregnant women

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We studied the arterial pressure response to maximal leg exercise in 15 healthy volunteers at 29 and 35 weeks' pregnancy and at 8 weeks post partum. Resting mean arterial pressure at 35 weeks' gestation was similar to the postpartum control value of 88 mm Hg (range 73 to 111), but it was reduced by 10% at 29 weeks' gestation. Voluntary maximal effort averaged 2470 N (range 1450 to 3030) in the postpartum period, was similar at 29 weeks, and was reduced by 13% at 35 weeks' gestation. Mean arterial pressure showed a linear increase with force to a median maximum value of 131 (104 to 159) mm Hg, or 49% above the resting value in the postpartum period. The pressure response was unaffected by pregnancy, but was inversely related to maximal force, i.e., the individual's capacity to perform isometric exercise. (Am J Obstet Gynecol 1992;166:538-42.)

Key words: Pregnancy, maximal exercise, isometric exercise, blood pressure

Pregnancy and muscular exercise require physiologic adaptations to increase blood flow to the regions of the body with increased metabolic activity: the uterus and the exercising muscles. The cardiovascular responses to pregnancy and to dynamic exercise include an increase in cardiac output, through increases in heart rate and stroke volume, with a reduction in peripheral vascular resistance. As a net result of these changes, systemic blood pressure usually falls slightly during pregnancy, whereas it increases mildly during dynamic exercise.[1] The adaptations are so effective that the combined demands of pregnancy and dynamic exercise do not appear to compromise the healthy fetus[1-3] nor affect the capability of healthy pregnant women to perform dynamic aerobic exercise.[3]

In contrast to dynamic exercise in which muscles undergo shortening or lengthening, isometric exercise does not result in muscle movement. Sustained muscle activity not only induces local vasodilatation but also muscle ischemia and mechanical obstruction to muscle perfusion.[4] Therefore, isometric exercise elicits a different cardiovascular response, with a more pronounced increase in arterial pressure than that during dynamic exercise. The mechanisms of these responses have recently been reviewed[5] and will be discussed in Comments. The magnitude of the response is dependent on the strength and duration of muscular contraction.[4]

The combined effect of isometric exercise and pregnancy has only been studied by means of handgrip exercise.[6-8] However, in handgrip exercise the active muscle mass is relatively small and the required force cannot be accurately determined. In the present longitudinal study we investigated the effects of pregnancy on the capability of the body to perform strenuous isometric exercise, and we determined blood pressure and heart rate responses to such exercise.

Material and methods

We studied 15 healthy women at 29 and 35 weeks of pregnancy and at 8 weeks post partum. All women were healthy, normotensive, and had uncomplicated singleton pregnancies, but they varied with regard to body weight and physical fitness. All women included in the study gave their informed consent. The study was approved by the Hospital and University Ethics Committee.

The women performed isometric exercise in an air-conditioned room kept at 21° C and at 60% humidity. Before each test we performed a routine physical and obstetric examination and monitored fetal heart rate (Corometrics, Fetal monitor 111, Wallingford, Conn.) to confirm the health of both individuals participating in the study. In addition, we determined body weight and leg length, from heel to trochanter major. The woman was placed on an ergometer designed for this study (Central Research Workshop, EUR, Rotterdam, the Netherlands). It consisted of a chair with a horizontal, adjustable footboard. The distance between the back of the chair and the footboard was adjusted to

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Table I. Effects of pregnancy on values at rest and at maximal isometric leg exercise

	Heart rate (beats/min)	*Blood pressure (mm Hg)*			*Force (N)*
		Systolic	*Mean*	*Diastolic*	
At rest					
29 weeks	92* (86-107)	112* (91-130)	79* (74-101)	68* (54-84)	—
35 weeks	90* (72-101)	119 (100-129)	87 (73-93)	74 (53-81)	—
Post partum	78 (60-94)	119 (102-135)	88 (73-111)	76 (61-93)	—
Maximum					
29 weeks	131* (108-163)	147 (133-173)	122 (101-145)	108 (92-126)	2400 (1470-3160)
35 weeks	122* (98-170)	145 (127-181)	123 (106-155)	108 (90-132)	2160* (1400-2800)
Post partum	119 (93-156)	153 (127-181)	131 (104-159)	115 (90-137)	2470 (1450-3030)

Median values with ranges in brackets.
*$p < 0.05$ compared with postpartum control values, $n = 15$.

90% of the length of the woman's legs and was kept constant between measurements within each individual. After 15 minutes of rest on the ergometer, the force exerted on the footboard was progressively and stepwise increased by 400 N per increment, until voluntary maximum effort was reached. Efforts of 45 seconds' duration were separated by 2 minutes of rest. The force exerted on the footboard was measured electronically with calibrated strain gauges and found to be accurate within 1% over the full range of measured forces. Heart rate and systolic, mean, and diastolic arterial blood pressures were measured oscillometrically (Dinamap 845, Applied Medical, Tampa, Fla.), a method validated for the observed range of measurements.[9] Measurements took 20 to 45 seconds to be completed and were initiated 2 minutes before and at the onset of exercise. During the exercise the volunteer was encouraged to continue to breathe normally and not to use her arm muscles.

Each woman underwent an initial test to get used to the experimental circumstances; the data obtained in this test were discarded. The Friedman and Wilcoxon ranked-sign tests were used to analyze differences between paired variables. Linear regression analysis was used to assess relationships between variables within each individual; the slopes and intercepts were treated as variables. A p value of <0.05 was taken as the level of significance.

Results

All 15 women remained healthy throughout the study period and were delivered of healthy infants. Median age at the time of delivery was 28 (range 24 to 34) years, gestational age 40 (37 to 42) weeks, and birth weight was 3.4 (2.6 to 3.9) kg.

All women were studied at 29 (26 to 32) weeks and 35 (33 to 37) weeks of pregnancy and 8 (6 to 10) weeks after delivery. Body weight was 72 (54 to 96) kg at 29 weeks, 74 (59 to 99) kg at 35 weeks' gestation, and 68 (50 to 93) kg 8 weeks post partum. Median leg length was 92 (78 to 98) cm. Six women were lactating at the time of the postpartum test. Because their responses did not differ significantly from those in the other women, we report the combined data.

The effects of pregnancy on values of heart rate and blood pressures at rest and at maximal exercise are summarized in Table I. Heart rate at rest was consistently increased during pregnancy above the median postpartum value of 78 beats · min^{-1}. Arterial blood pressures at rest were significantly below postpartum values at 29 weeks' gestation, but they were not markedly different at 35 weeks of pregnancy. Maximal force was not significantly different from the median postpartum value of 2470 N at 29 weeks' gestation, but at 35 weeks' gestation maximal force was significantly lower than at 29 weeks and post partum, by −3% to −13%, in 11 and 13 of the 15 women, respectively. Heart rate during maximal isometric exercise in pregnancy was significantly elevated by 3% to 10% above the postpartum value of 119 beats · min^{-1}, whereas systolic, mean, and diastolic pressures were not significantly different among the three periods.

Heart rate and systolic, mean, and diastolic pressures were linearly related to force and to each other (median $r = 0.96$, range 0.70 to 0.99). This is shown for heart rate and mean arterial pressure in Figs. 1 and 2. Pregnancy did not significantly affect the slope of any of the lines. In contrast, Fig. 2 demonstrates that the position of the mean arterial pressure-heart rates lines are affected by pregnancy, as are the values of heart rate and blood pressure at rest.

The individual's capacity to perform isometric leg exercise was unrelated to body weight and to maximal values of heart rate or arterial pressures. However, the systolic, mean, and diastolic arterial pressure responses were inversely related to performance. The mean arterial pressure response to a change in force can be expressed as Δmean arterial pressure/Δforce = $-0.007 \times$ maximal force (kN) + 35 ($r = 0.70$, $p = 0.004$) and varies from 24 mm Hg/kN at a maximal force of 1.5 kN to 13 mm Hg/kN at 3 kN. The relationship is unaffected by pregnancy.

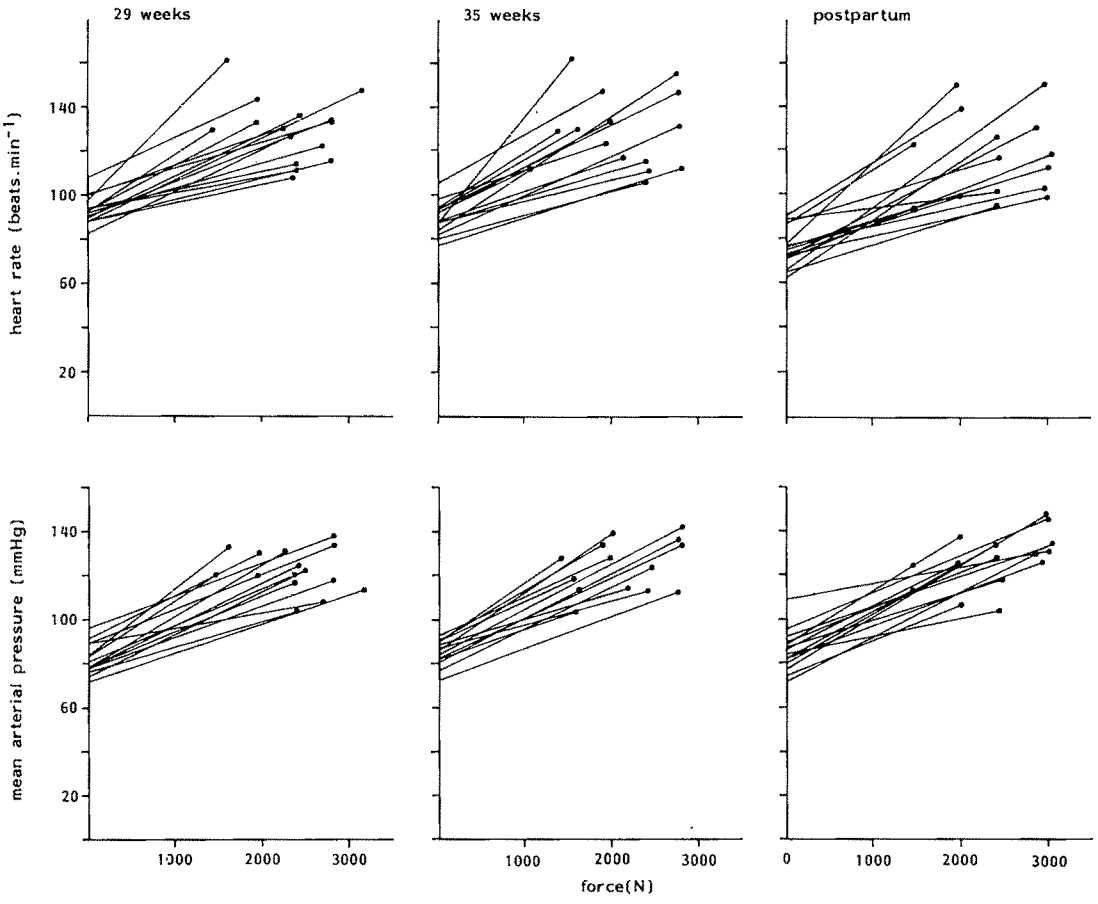


Fig. 1. Individual regression lines representing heart rate and mean arterial pressure responses to increasing levels of isometric exercise at 29 and 35 weeks' gestation and at 8 weeks post partum, $n = 15$.

Comment

The first report on isometric exercise in the obstetric literature concerns a study in which it was proposed that isometric handgrip exercise could serve as a simple and reliable test for the prediction of pregnancy-induced hypertension.[6] That study has been criticized,[10] and in a more recent study no difference in response to handgrip exercise was observed between women with pregnancy-induced hypertension and normotensive women with regard to blood pressure, heart rate, cardiac output, and catecholamine concentrations.[8]

The present study is an effort to study whether pregnancy affects the capability of the body to perform strenuous isometric exercise. We realize that the study has its limitations. First, the study does not have the statistical power to conclude that there is really no difference in pressure response between the pregnant and the postpartum state. However, on the basis of our results a physiologically important difference in response seems unlikely. Second, the postpartum state may not represent the true nonpregnant state. This is true, but in studies in dynamic exercise similar results were obtained at 7 weeks and 6 months post partum. Third, the oscillometric measurement of arterial pressures has not been validated for the use in exercise studies. However, the method has been validated for the observed range of pressures under resting conditions,[9] and we made sure that the volunteers did not use their arm muscles and continued to breathe during isometric leg exercise. Any motion error should have affected pregnant and nonpregnant women alike. We found measurements of maximal force and blood pressures to be reproducible within 10% in three nonpregnant individuals. Fourth, isometric exercise of longer duration may elicit a different response. This point will require further study.

The capability to perform dynamic exercise is unaffected by pregnancy, as both maximal oxygen uptake and maximal power remain the same.[3] The results of the present study indicate that pregnancy does also not affect the capability to perform maximal isometric leg exercise at 29 weeks' gestation. The finding that maximal force was on average 10% lower at 35 weeks than at 29 weeks might be explained by a reduction in the

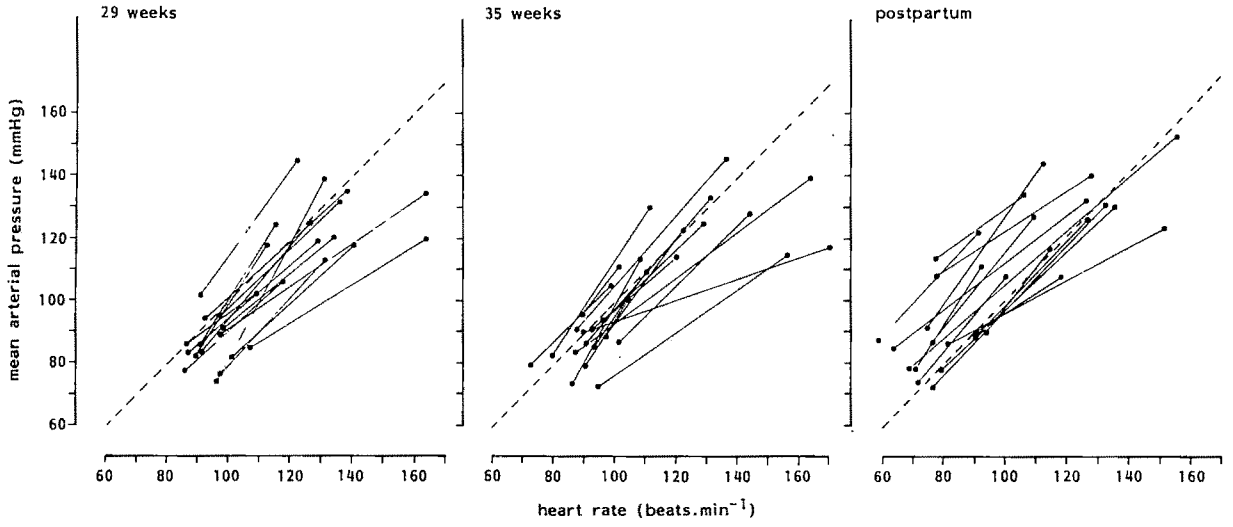


Fig. 2. Individual regression lines representing increase in mean arterial pressure with heart rate during isometric exercise at 29 and 35 weeks' gestation and at 8 weeks post partum, $n = 15$.

supply of oxygen and nutrients to the isometrically exercising muscles, by a decrease in the functional reserve to anaerobic exercise, or by reaching voluntary maximal exercise at an earlier stage. Because the increase in mean arterial pressure with force at 35 weeks' was not significantly different from that in the two other periods, as shown in Fig. 1, the first two possibilities seem unlikely. In dynamic exercise, maximal effort can be determined objectively by the measurement of maximal oxygen uptake,[1] whereas in isometric exercise maximal effort is determined by the subjective feeling of the volunteer. It is conceivable that in some women at 35 weeks' the mechanical discomfort of the protruding abdomen has contributed to reaching a feeling of maximal effort sooner than at 29 weeks' gestation or post partum.

The rise in arterial pressure during isometric exercise may result from an increase in cardiac output, systemic vascular resistance, or both. It is thought that heart rate increases during isometric exercise mainly by central command and vagal withdrawal, whereas blood pressure rises initially by both central command and muscle mechanoreflexes.[5] Later, after approximately 1 minute of exercise, the muscle chemoreflex induces sympathetic nervous activity that affects catecholamine concentrations and systemic vascular resistance.[5] If this concept is true, it seems likely that the increase in blood pressure in our study was caused by increase in cardiac output rather than by a rise in systemic vascular resistance, because periods of exercise were restricted to 45 seconds.

The quotient of mean arterial pressure and heart rate equals the product of stroke volume and systemic vascular resistance. Mean arterial pressure/heart rate is lower at rest at 29 weeks' gestation than it is post partum, as shown in Fig. 2. This is in agreement with the observation that the decrease in systemic vascular resistance during pregnancy is more pronounced than the increase in stroke volume.[11] The constancy of the quotient of mean arterial pressure and heart rate during isometric exercise, as shown in Fig. 2, demonstrates that the blood pressure response is proportional to the increase in heart rate. If the product of stroke volume and systemic vascular resistance during isometric exercise is almost constant and if exercise of short duration does not affect vascular resistance, as was discussed above, stroke volume must also be virtually constant. This would imply that the increase in cardiac output during isometric exercise of short duration is primarily caused by vagal withdrawal through central command, as suggested in the literature.[5]

In spite of altered values of heart rate and arterial pressures at rest, pregnancy does not seem to affect the pressure response to either dynamic[1] or isometric exercise.

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Transvaginal ultrasonographic quantitative assessment of accumulated cul-de-sac fluid

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Preoperative quantitative assessment of pelvic fluid is an important diagnostic tool in clinical decision making. In this study, we used high-frequency transvaginal ultrasonography in 10 healthy women to assess both the correlation between various amounts of fluid installed in the cul-de-sac and ultrasonic imaging and to determine whether correct estimation of fluid volume can be reached. No fluid could be ultrasonically detected when the volume was <35 to 40 ml. Between 35 and 100 ml there was a clear image of accumulated fluid in the cul-de-sac with good correlation between volume and image, whereas with larger amounts no clear correlation between the amount of fluid introduced and size of fluid area visualized by ultrasonography was possible. Transvaginal ultrasonography may enable quantitative evaluation of certain volumes of pelvic fluid, although amounts <35 ml cannot be visualized. (AM J OBSTET GYNECOL 1992;166:542-4.)

Key words: Cul-de-sac, pelvic fluid, transvaginal ultrasonography

During the past 5 years transvaginal ultrasonography has increasingly gained acceptance in obstetrics and gynecology as a result of its higher resolution. Its use thus provides a more accurate diagnosis of pelvic masses and early detection of extrauterine pregnancies. Although some free fluid is normally present in the female pelvis, especially in midcycle,[1] increased amounts may indicate pathologic conditions, such as bleeding from a tubal pregnancy or a ruptured cyst. Previous reports have indicated that quantitation of free pelvic fluid is possible[2]; however, no direct studies under controlled conditions have been reported. The current study describes the use of transvaginal ultrasonography in quantifying free pelvic fluid volumes.

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Patients and methods

Transvaginal ultrasonography with a 6.5 MHz transducer probe (Elscint Ltd., Haifa) was performed on 10 healthy women during diagnostic laparoscopy for infertility. In all women the results of laparoscopy were negative for any pelvic pathologic conditions. After completion of the laparoscopic survey of the pelvic organs, all free pelvic fluid was aspirated with the patients in reverse Trendelenburg position. The patients were then placed in supine lithotomy position, and a plastic 18-gauge Intracath catheter was introduced through the abdominal wall into the cul-de-sac. The carbon

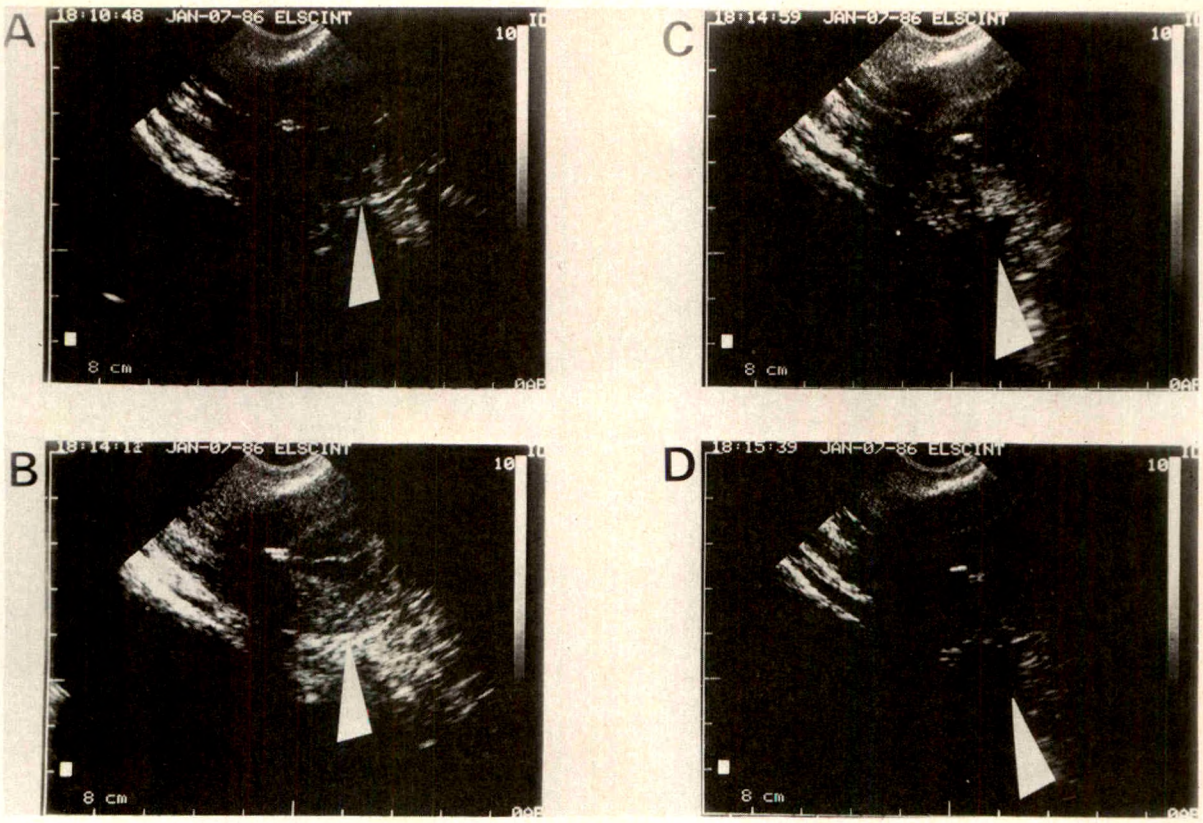


Fig. 1, A to D. Serial images of cul-de-sac with increasing amounts of fluid. **A,** 0 ml; **B,** 20 ml; **C,** 35 ml; **D,** 40 ml.

Table I. Cul-de-sac fluid triangle size (millimeters) measured at different amounts of injected fluid

Patient No.	*Total amount of fluid injected*					
	0-30 ml	*35-40 ml*	*50 ml*	*60 ml*	*80 ml*	*100 ml*
1	0 × 0 × 0	7 × 6 × 7	10 × 9 × 8	13 × 12 × 15	20 × 22 × 16	26 × 22 × 21
2	0 × 2 × 0	7 × 8 × 6	11 × 9 × 9	14 × 13 × 13	20 × 21 × 15	26 × 23 × 20
3	0 × 0 × 3	6 × 7 × 8	11 × 8 × 8	13 × 14 × 15	18 × 19 × 17	24 × 22 × 22
4	3 × 0 × 1	8 × 8 × 6	11 × 9 × 9	13 × 13 × 15	20 × 22 × 17	26 × 25 × 22
5	0 × 0 × 1	7 × 6 × 7	10 × 10 × 10	12 × 12 × 16	22 × 19 × 13	25 × 24 × 22
6	0 × 0 × 0	6 × 7 × 7	10 × 8 × 9	14 × 14 × 14	19 × 20 × 16	25 × 22 × 21
7	0 × 0 × 0	8 × 7 × 7	10 × 9 × 9	13 × 12 × 15	20 × 21 × 15	24 × 21 × 22
8	0 × 0 × 0	8 × 8 × 8	11 × 10 × 8	12 × 13 × 17	21 × 20 × 14	26 × 22 × 21
9	0 × 0 × 0	6 × 7 × 6	10 × 8 × 9	13 × 14 × 15	20 × 18 × 15	24 × 21 × 20
10	0 × 0 × 0	7 × 6 × 8	10 × 10 × 9	12 × 13 × 16	21 × 20 × 15	25 × 21 × 23

dioxide gas was evacuated from the peritoneal cavity and the laparoscope was then removed. Normal saline solution was introduced into the cul-de-sac through the catheter at increments of 5 to 10 ml, up to a maximum of 150 ml. After each incremental addition, an ultrasonographic image was obtained by directing the beam longitudinally at the midline of the uterus and cul-de-sac, and the image was recorded. At the end of the procedure, the pelvic fluid was reaspirated through the same catheter.

Results

Good-quality images were obtained for all patients. A representative set is shown in Figs. 1 and 2. In all cases fluid up to 30 ml was not seen in the cul-de-sac (Figs. 1 and 2). At 35 to 40 ml a small triangle of fluid, measuring 7 × 7 × 7 mm (± 1 mm), was observed under the most dependent part of the uterus. The size of the triangle increased to 9 × 9 × 10 mm (± 1 mm) when 50 ml was introduced and to 13 × 13 × 15 mm (± 2 mm) when 60 ml was introduced. When 80 ml was

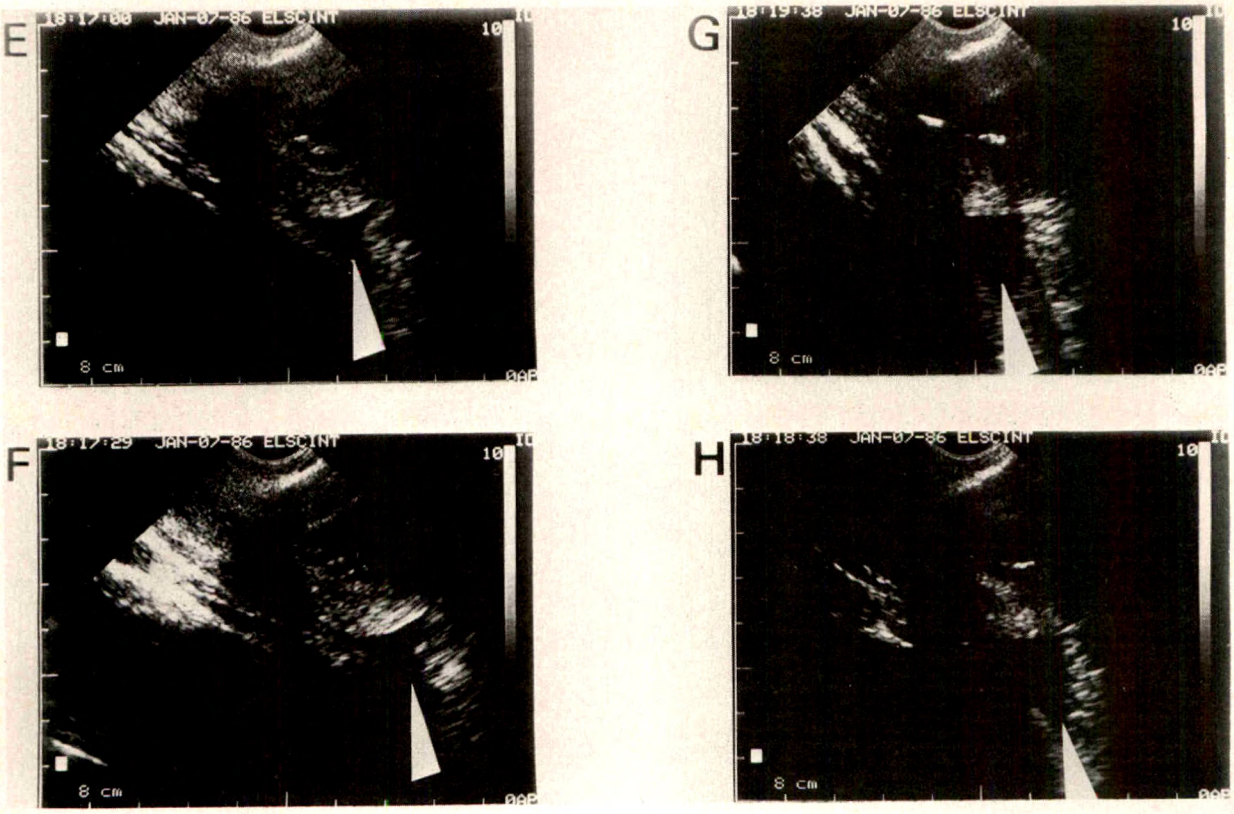


Fig. 1, E to H. Serial images of cul-de-sac with increasing amounts of fluid. **E,** 50 ml; **F,** 60 ml; **G,** 80 ml; **H,** 100 ml.

placed in the pelvis, the triangle enlarged to 20 × 20 × 15 mm (±2 mm). At 90 to 100 ml the fluid-filled area increased substantially and included the nondependent area of the cul-de-sac (Table I). With larger amounts of fluid, no clear delineation of the fluid-filled area was possible; however, increased amounts of fluid were observed. At the conclusion of the procedure, it was possible to aspirate almost all the injected fluid from the cul-de-sac, indicating that all of the fluid was indeed accumulated there.

Comment

Accumulation of fluid in the cul-de-sac may be indicative of a ruptured cyst or ascitic fluid (clear fluid), a ruptured corpus luteum cyst or ectopic pregnancy (blood), pelvic inflammatory disease, or perforated viscous fluid (pus). Because small amounts of fluid can normally be present in the cul-de-sac, it is important for the clinician to be able to correctly assess the amount of fluid. Timor-Tritsch et al.[2] have previously commented on this subject; however, they assessed the presence of fluid in the cul-de-sac during laparotomy, not seeking a correlation between fluid volume and the ultrasonographic image. As shown in this report, our patients were in supine position with closed intact abdominal walls after the removal of the previously introduced gas. All these measures were taken to achieve conditions as similar to normal scanning as possible. Any amount of fluid in the pelvis <35 ml could not be ultrasonographically detected. This is in contrast to the previous report stating that "five ml was easily seen."[2] The reason for this discrepancy is unclear; however, no data were provided in the previous report as to patients' tilt or the scanning plane. At an accumulation between 35 and 100 ml, we found a direct correlation between the size of the fluid area on the ultrasonographic image and the amount of fluid introduced into the pelvis. Accumulation of >100 ml was easily detected, but could not be quantified.

Thus it is possible to correctly assess the amount of pelvic fluid by transvaginal ultrasonography. We have been able to demonstrate this in several cases where transvaginal ultrasonography was performed before diagnostic laparoscopy and aspiration of measured amounts of pelvic fluid (Rosen DJD. Unpublished data).

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Comparison of thermal injury zones in loop electrical and laser cervical excisional conization

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Thirty cervical conization specimens obtained by thin-loop electric excision were compared with 30 carbon dioxide laser excisional cone specimens. Zones of thermal artifact were measured with a precise stage micrometer technique calibrated to a Zeiss standard. The mean depth of coagulation at the ectocervical margin measured 0.187 and 0.164 mm for thin loop and laser, respectively. Thermal artifact at the endocervical margin was greater with electric loop (0.295 mm) than with superpulsed carbon dioxide laser (0.137 mm). The zones of thermal injury induced by both electric loop and laser made no significant impact on the interpretation of margin adequacy. (Am J Obstet Gynecol 1992;166:545-8.)

Key words: Loop electric conization, carbon dioxide laser, thermal injury

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Recent reports of large-loop diathermy to perform outpatient conization for the treatment of cervical intraepithelial neoplasia have appeared in the peer review literature.[1-4] This method of excision combines the advantages of simplicity, hemostasis, and the retrieval of a pathologic specimen with economy. The latter may be exemplified by considering that women who have

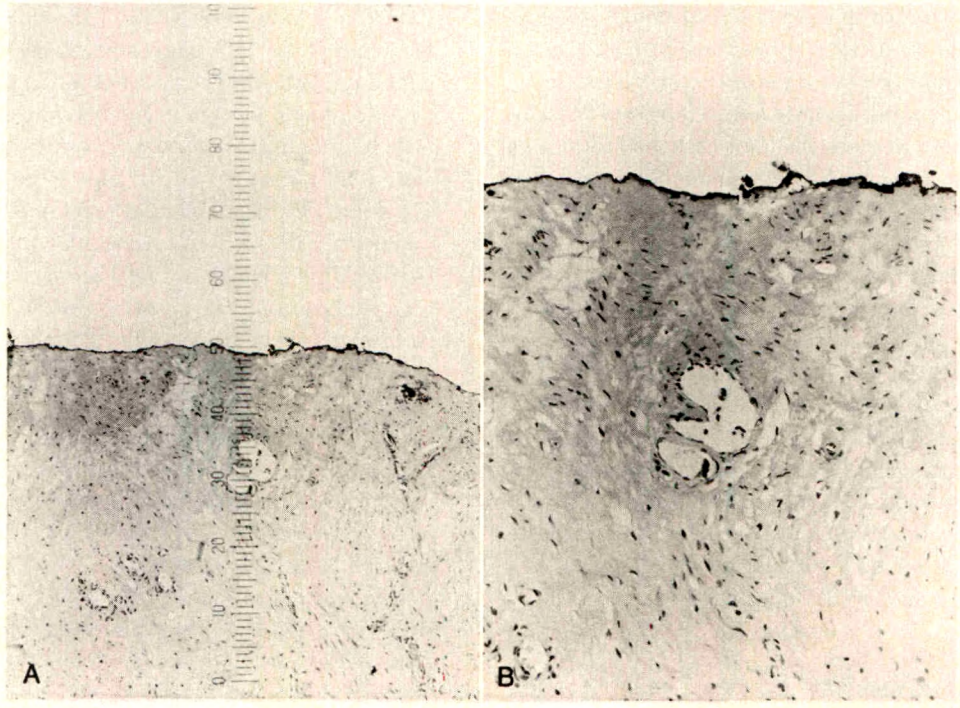


Fig. 1. A, Ectocervical margin of loop electric cone specimen. Stage micrometer is aligned perpendicularly with specimen. (Original magnification ×40.) **B,** Zone of thermal injury can be divided into small zone of carbonization and desiccated-coagulated zone beneath. (Original magnification ×100.)

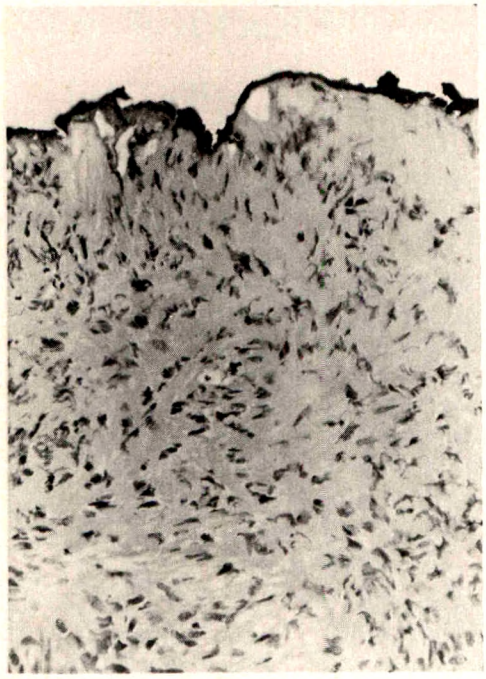


Fig. 2. Total zone of thermal injury measures 0.230 mm. (Original magnification ×200.)

been screened cytologically may undergo a colposcopic examination with accurate identification of the abnormal transformation zone followed by loop electric excisional conization, which provides both histologic diagnosis and therapy in one sitting. Although this therapeutic program at first appears to be ideal, certain concerns should be considered. The histologic quality of the specimen, particularly at the resection margins, is vitally important to determine whether all the disease has been removed and to determine the severity of disease. If thermal artifact makes this determination impossible, then this alone would constitute a major disadvantage of the technique. In the last decade the carbon dioxide laser became an accepted alternative to knife conization because of its precision and hemostatic benefits and because it eliminated the need for sutures. The literature contains descriptions of large numbers of laser conization operations whose specimens showed highly acceptable margins and demonstrated a slight degree of thermal injury to neighboring tissue.[5-7] This article concerns itself with prospectively comparing and accurately quantitating the zones of irreversible thermal injury created by the loop electric cone and the carbon dioxide laser cone.

Material and methods

Thirty loop electric excisional conization specimens were obtained from women with cytologic and colposcopically directed biopsy evidence of cervical intraepithelial neoplasia. Another 30 excisional conization samples emanated from women with cervical intraepithelial neoplasia diagnosed in the outpatient department who underwent laser excisional conization. Both laser and electric loop excisions were performed by M.S.B. or one of the pelvic surgery fellow coauthors. All cone specimens were fixed in formalin, cut, blocked, sectioned, and stained with hematoxylin and eosin.

Table I. Mean depth of thermal injury in excisional conization

Technique	*Ectocervical margin (mm)*	*Endocervical margin (mm)*
Loop electric	0.187	0.295
Carbon dioxide laser	0.164	0.137

The specimens were examined by M.S.B. with an Olympus Vanox T Photomicroscope (Olympus, Newtown, Pa.) equipped with a built-in stage micrometer and floating stage. Before the sections were examined, the stage micrometer was calibrated to a 1 mm standard (Carl Zeiss, Thornwood, N.Y.) subdivided into 0.01 mm segments. The calibration was made with ×4 and ×10 objectives. Margins were measured by orienting the stage to effect a perpendicular measurement, initiating the measurement at the outer carbonized zone and finishing at the beginning of normal stromal or epithelial tissue. Three measurements were performed for each section and averaged. A total of 1200 sections were studied.

An ERBE model 450 (ERBE, USA, Mission Viejo, Calif.) or a Valley Lab Force 2 (Boulder, Colo.) high-frequency (350-750 kHz), low-voltage electrosurgical generator with a 400 W rated output at 350 Ω resistance with automatic voltage regulation during cutting was used for all loop excisions. Five predetermined settings were available with the Erbe unit. The Valley Lab unit was set to deliver 50 W of power. A setting that used a four fifths cut to one fifth coagulation blend was used (setting 200 to 500 V) for the 30 conizations performed with this equipment. The loops used to perform the conizations ranged from 15 to 10 mm in height (measured from the top of the loop to the insulated Y connection of the probe yolk). The wire electrodes measured 0.2 mm in diameter. Conizations were performed by placing the loop perpendicularly to the cervix at either the 12 or 6 o'clock position with approximately a 3 mm margin around the atypical transformation zone, activating the cutting button on the handle of the probe, and allowing the wire to cut into the cervix to the desired depth. Next the loop was swept across the base of the tissue with very slight pressure while the cutting button was depressed, i.e., allowing the speed of the vaporization to determine the velocity of the sweep and subsequent finishing scoop action of the operator's hand.

A Lasersonics model 500 Z (Heraeus Lasersonics, Milpitas, Calif.) carbon dioxide laser rated at 60 W power and equipped with a micromanipulator was cou-

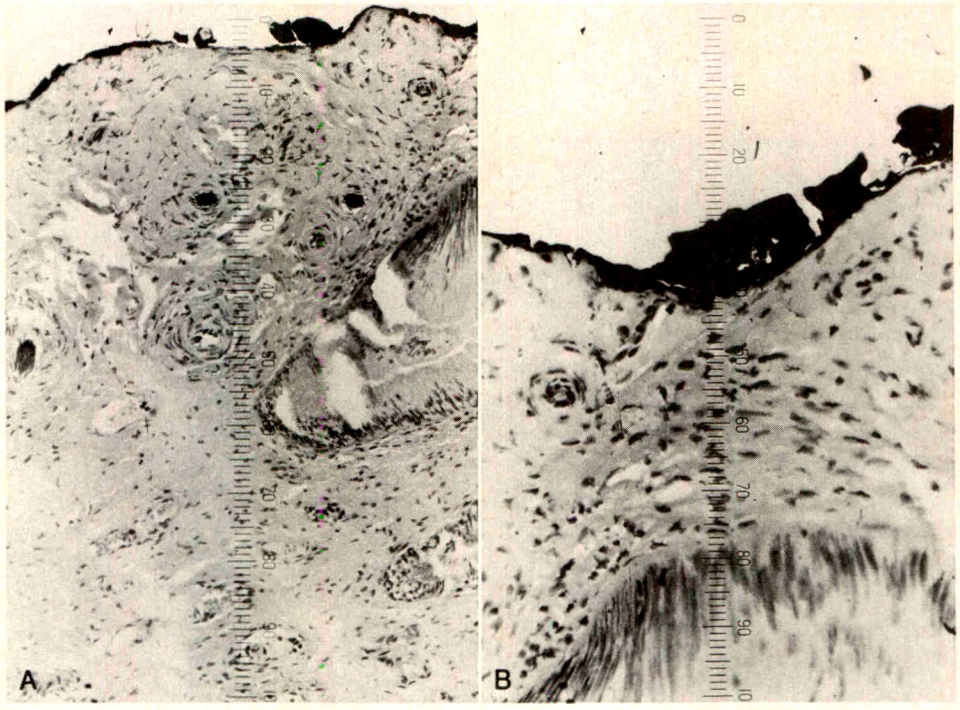


Fig. 3. A, Endocervical margin of loop electric excisional cone shows more extensive zone of thermal injury. Note vascular thrombosis and endocervical cleft distortion. (Original magnification ×100.) **B,** Detail of endocervical margin with 0.390 mm coagulation artifact. (Original magnification ×200.)

pled to a Zeiss OPM 1-H colposcope. The 30 laser conizations were carried out with 40 W of power and a 0.5 mm spot according to methods previously described.[5] The endocervical margin was cut off with a superpulsed beam emitting 500 pulses/sec, 0.5 millisecond pulse width, with an average power of 23 W.

Results

Measurement of sections obtained from loop excisions revealed two zones of thermal injury at the ectocervical and endocervical margins (Table I): (1) a small zone of carbonization and tissue debris (Fig. 1) and (2) a larger zone of coagulation necrosis and vascular coagulation (Fig. 2). The latter could be easily defined from normal stromal tissue by its deeper pink color and its denser appearance (a secondary effect of desiccation). The range of total thermal injury was 0.91 to 0.300 mm with a mean of 0.187 mm.

The thermal injury zone was greater in both range and mean at the endocervical margin (Fig. 3). The range of thermal injury extended from 0.190 to 0.455 mm with a mean thermal injury zone of 0.295 mm.

Laser conization specimens demonstrated identical zones of thermal injury, i.e., carbonized zone and desiccation-coagulation zone. The ectocervical margins total from 0.091 to 0.210 mm with a mean zone of 0.164 mm. The endocervical margin's zone of thermal injury was smaller than those observed with the electric loop, ranging from 0.090 to 0.230 mm with a mean of 0.137 mm (Fig. 4). However, this small difference made no impact on the interpretation or adequacy of the margin.

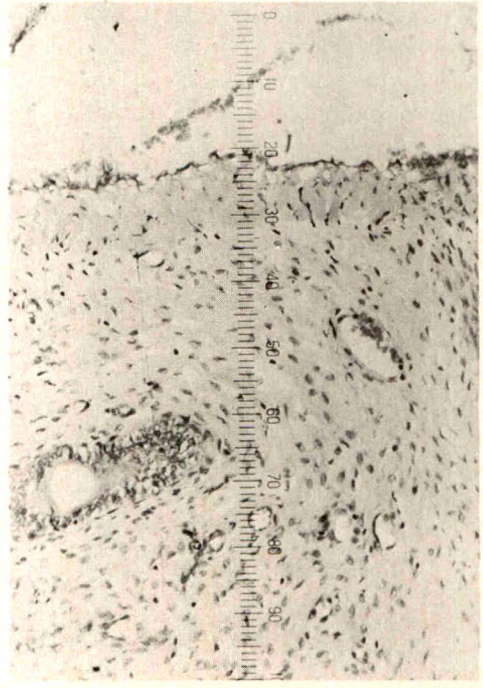


Fig. 4. Superpulsed laser endocervical margin measures 0.143 mm with small carbonization layer. (Original magnification ×100.)

The carbonized zones were virtually identical in both groups, measuring 0.013 to 0.026 mm.

A major difference was noted in specimens taken with the electric loop measuring <5 mm deep. The zone of thermal injury in these instances was extensive, i.e., measuring up to 0.560 mm. The endocervical mucosa particulary showed extensive thermal artifact at the margin of resection. This degree of thermal injury was not produced with superpulsed laser beams, which again showed thermal injury measuring 0.090 mm to 0.230 mm.

Comment

Excisional cones obtained with high-frequency, low-voltage, thin wire loops created wounds that were virtually identical to those obtained with the carbon dioxide laser. The zones of thermal injury were acceptably small and did not interfere with the histopathologic interpretation at either the ectocervical or endocervical margins. From this study the method of tissue ablation appears to be similar with either electric loop or laser modality, i.e., thermal energy creates temperatures exceeding 100° C, resulting in vaporization of tissue. Lesser temperatures resulted in neighboring tissue undergoing desiccation and coagulation with resultant tissue distortion (thermal artifact). At the setting used in this study voltages between 200 and 500 were produced at the interface of the electrode (wire loop) and the tissue, creating electric arcs ideal for vaporization and associated with a low degree of coagulation. When voltage exceeded 500, more extensive coagulation was created with increased thermal artifact. Therefore the narrow band of 200 to 500 V is the acceptable range for electric excisional conization operations.

The laser wounds demonstrated in this study were obtained at power densities of approximately 16,000 W/cm^2. Less thermal damage was obtained at margins where superpulsed beams were used because peak powers were extremely high (250 to 400 W) and pulsing allowed thermal relaxation of the tissues. With higher average superpulse powers, even less thermal injury might be anticipated, and over a large range of power settings, repetition rates, and pulse widths the zone of thermal injury in such instances would clearly vary directly with the duty cycle of the laser so long as that cycle remains <25%.

This study demonstrated that thin-loop high-frequency, low-voltage electric surgery and high–power density carbon dioxide laser produced similar wounds in excised human cervical tissue. The advantages of the electric thin loop relate to its simplicity and speed.

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Nematode infections: Is it wise to withhold medical treatment during pregnancy?

I. Biliary complications

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Appropriate medical treatment should be provided in ascariasis because this benign infection could cause unusual and dangerous complications in pregnancy and may increase maternal-fetal morbidity and mortality. (AM J OBSTET GYNECOL 1992;166:549-50.)

Key words: Nematodes, ascariasis, antiparasitic drugs, gallbladder disease

Ascaris lumbricoides is the most common helminthic infection in humans, with an estimated prevalence of 1 billion infections, with >4 million of these in the United States. Adult worms are known to inhabit the lumen of the small intestine (jejunum and ileum) and have a short life span of approximately 10 to 24 months. Their tropism and, when conditions are unfavorable, migratory tendency toward anatomic orifices make possible the invasion of a majority of organs in the body, resulting in extraintestinal forms of the parasite. Furthermore, this invasion has been considered by others[1] as responsible for coadjuvant illness, such as biliary lithiasis.

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Case report

A 24-year-old Hispanic woman, gravida 1, para 0, was evaluated by her obstetrician during the twelfth

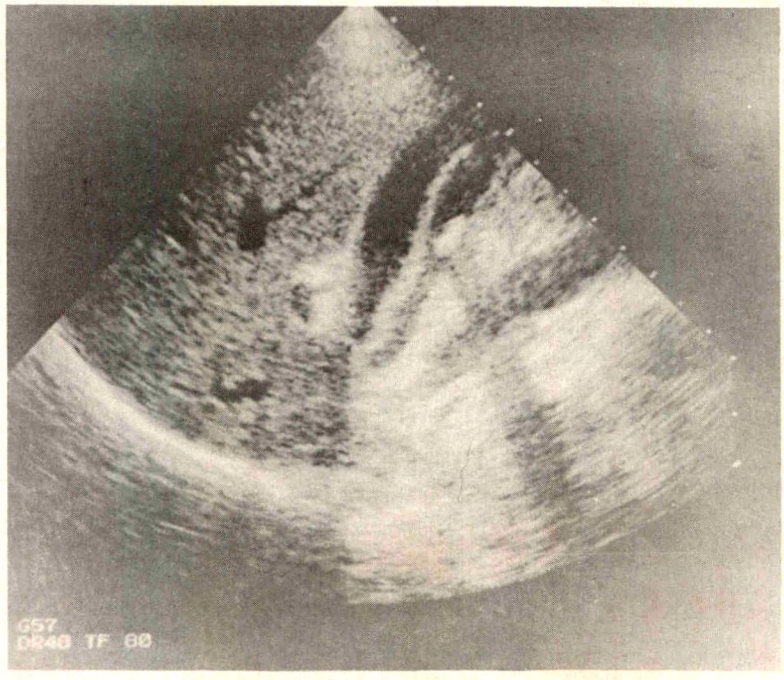


Fig. 1. Sonogram showing adult *A. lumbricoides* worm along with biliary lithiasis in a pregnant patient at 20 weeks of gestation.

week of gestation. Stool test showed the presence of *A. lumbricoides* eggs. Because of the corollary that "parasitic infections should not be treated during pregnancy," a couple of months later she came to the emergency room complaining of severe upper-right-quadrant pain, nausea, and vomiting. Abdominal ultrasonography revealed the presence of a large adult *A. lumbricoides* worm along with gallbladder stones (Fig. 1), which were obstructing the cystic duct. She was taken to the operating room where, through cholecystectomy, the diagnosis was confirmed. Two more organisms were found after medical treatment with piperazine citrate, 75 mg/kg daily for 2 days. A control stool test showed no further infection. No adverse effects were seen in the healthy male newborn delivered 18 weeks later.

Comment

A number of drugs are effective in the treatment of roundworm infections (ascariasis). Pyrantel pamoate (Antiminth) is highly effective with a single dosage regimen of 11 mg/kg. Piperazine salts (Antepar, Multifuge) are still widely used. Thiabendazole (Mintezol) is generally less effective than pyrantel pamoate or piperazine. The broad-spectrum antihelminthic mebendazol (Vermox) is effective against *A. lumbricoides*, but multiple doses are required (100 mg twice a day for 3 days) to achieve a high cure rate. Treatment failures with this drug have been reported previously. A 3-day regimen of pyrantel pamoate with a single daily dose has been suggested for treatment failure with mebendazol.

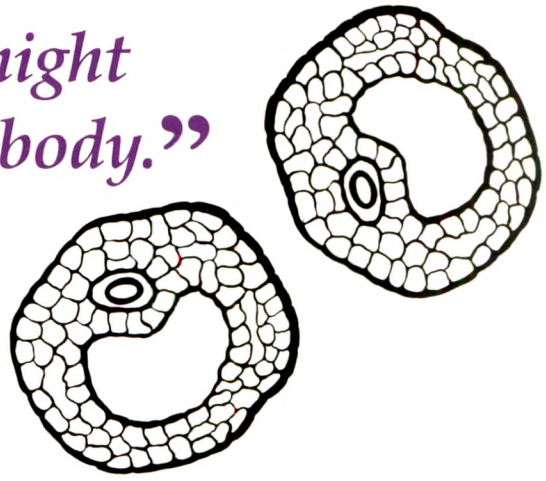
Heinonen et al.[2] provided a large sample of pregnant patients in whom piperazine citrate was prescribed. No evidence was found to suggest a relationship with fetal malformations. Experience with mebendazol and pyrantel pamoate in human pregnancies is not available, and recommendation to withhold their use is based on results in animal studies.

In conclusion, ascariasis in pregnancy may not be as benign as expected; therefore these infections should always be treated because they have the potential for causing serious complications in spite of the fact that most patients are asymptomatic. Antiparasitic drugs, such as pyrantel pamoate and piperazine citrate, have not shown adverse effects on the fetus according to our brief experience (unpublished data).

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"Another Saturday night and we ain't got nobody."



DEMULEN® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Where protection begins

The physician should become familiar with the prescribing information (labeling) for this product, which discusses reported risks, such as thromboembolic disorders (including strokes and heart attacks), risk of estrogen dose, and hepatic lesions, as well as the need to monitor patients for early symptoms of any diseases or medical conditions, so use can be discontinued when appropriate.

Please see adjoining page for a brief summary of prescribing information.

The OC innovator SEARLE

DEMULEN® 1/35

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Demulen® 1/35: Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients. **Demulen® 1/50:** Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients.

Indication and usage: For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk.* Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen.

Contraindications: Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products.

Warnings:

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE); British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke, increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women-Year

	Aged 30-39	*Users*	*Nonusers*	*Aged 40-44*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. Latter group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myocardial ischemia, occurs with high progestational activity. Consider amount of both steroids in choosing OC. *Persistence of Risk after OC discontinuation was reported for* circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs.

Risk is: A—very high; B—high; C—moderate; D—low. Age	<30	30-39	≥40
Heavy smokers (≥15/day)	C	B	A
Light smokers (<15/day)	D	C	B
Nonsmokers, no predispositions	D	C,D	C
Nonsmokers, with predispositions	C	C,B	B,A

Be alert to earliest manifestations of TE disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer with combination or progestogen-only OCs. Studies show no increased breast cancer with OCs or estrogens. However greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors and in various subgroups. Decrease in benign breast tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia. CIN, erosion, and carcinoma in long-term users. Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, etc.* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur with OC use. **5.** *Use in or immediately preceding pregnancy; birth defects; malignancy in offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES-exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,000 births compared to 7.8 for those unexposed (P<.05). Female hormone exposure in utero was associated with testicular and ovarian cancer. More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed, rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, stop OC and apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. Bile duct cancer has been reported. **7.** *Carbohydrate and lipid metabolism:* Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides, total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but increases with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. **10.** *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a higher-estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. **12.** *Breast feeding:* OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III; increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake; unaltered free T^4; decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydial and gonorrheal prevalence is increased; do not assume OC protects against chlamydial PID. HIV seropositivity was associated with OC use. *Information for patients.* See patient labeling. *Drug interactions.* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease; myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease. Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding; liver cancer; congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nausea, vomiting, gastrointestinal symptoms; BTB, spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after use; infertility post-OC; edema; chloasma or melasma, which may persist post-OC; breast changes (tenderness, enlargement, secretion); weight changes; change in cervical erosion and secretion; endocervical hyperplasia; lactation decrease; cholestatic jaundice; migraine; enlarged uterine leiomyomata; allergic rash; mental depression; reduced carbohydrate tolerance; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, cataracts; libido changes; chorea; appetite changes; cystitis-like syndrome; headache; paresthesia; nervousness; dizziness; auditory disturbances; rhinitis; fatigue; backache; hirsutism; scalp hair loss; erythema multiforme or nodosum; hemorrhagic eruption; herpes gestationis; hidradenitis suppurativa; hemolytic uremia; malignant hypertension; impaired renal function; acute renal failure, sometimes irreversible; itching; vaginitis; porphyria; PVCs; abnormal EKG; pulmonary hypertension; TTP; anemia; pancreatitis; hepatitis; colitis; Crohn's disease; gingivitis; dry socket; lupus erythematosus; rheumatoid arthritis; pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use; malignant melanoma; endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use and is available on request.**

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Composite assessment of gestational age: A comparison of institutionally derived and published regression equations

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OBJECTIVES: This study was undertaken to evaluate (1) the applicability of previously reported regression equations for gestational age assessment to our patient population in Pittsburgh and (2) whether the addition of radius length or its substitution for one of the other fetal measurements could improve the prediction of gestational age.

STUDY DESIGN: Five fetal parameters—biparietal diameter, head circumference, abdominal circumference, femur length, and radius length—were measured in a prospective cross-sectional study of gestational age assessment in 265 pregnant women between 13 and 43 weeks' gestation.

RESULTS: The regression equation derived for each fetal parameter was found to be quite similar to previously published reports. Although the coefficient of determination was only minimally affected, the variability improved with the institutionally derived regression equation.

CONCLUSION: The incorporation of several fetal measurements into a composite assessment of gestational age generally enhances the accuracy of the prediction. The addition of a second long bone to a gestational age assessment does not significantly improve results. (Am J Obstet Gynecol 1992;166: 551-5.)

Key words: Estimation of gestational age, multiple fetal growth parameters

The biparietal diameter was one of the first fetal parameters used to assess gestational age.[1] To this day, it remains the standard against which all other fetal biometric data for predicting gestational age are compared.[2-5] In general, the confidence intervals for any fetal measurement increase with advancing gestation. With respect to the biparietal diameter, for example, the maximum variability exceeds 3.0 weeks during the last month of pregnancy.[6] Hadlock et al.[7] sought to improve the estimation of gestational age in the third trimester by combining the data from two or more of the following fetal measurements: biparietal diameter, head circumference, abdominal circumference, and femur length. A simple averaging technique was used in which equal weight was given to each parameter measured. For example, when all four parameters were used, the individual estimates of gestational age on the basis of each parameter were added and then divided by four to obtain a composite assessment of gestational age. This method not only improved the correlation coefficient with gestational age but also reduced maximum variability. In a subsequent study by Hadlock et al.,[8] multivariate regression was used to determine the independent contribution of each of these fetal biometric parameters to the determination of gestational age. Although the multivariate regressions provided better estimates (i.e., reduced variability) than univariate regressions, the results were not significantly different from those obtained with a simple averaging technique.[8]

The current study represents an extension of the work by Hadlock et al.,[8] stemming from two considerations. First, it is possible that, although statistical predictions of gestational age may be quite accurate for the population from which they are derived, they may not be reliably applied to a patient group from a different geographic region or institutional setting. Thus one goal of the present study is to assess the applicability of the regression equations derived by Hadlock et al.[8] from their Houston data to our patient population at Magee-Womens Hospital in Pittsburgh. Second, when Hadlock et al.[8] published their work on the composite assessment of gestational age, they evaluated the four fetal parameters most commonly measured at that time. Several additional fetal measurements are now available. For example, we have recently assessed radius length as a predictor of gestational age.[9] A second goal of the study is to determine if the addition of radius length, or its substitution for one of the standard fetal measurements mentioned above, could improve the prediction of gestational age.

Material and methods

The study group consisted of 265 consecutive women who were delivered at Magee-Womens Hospital, Pitts-

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Table I. Houston regression equations as applied to Pittsburgh data

Fetal measurements (cm)	*Houston regression equation*	*SD (wk)*	
		Houston data	*Pittsburgh data*
BPD	$GA = 9.54 + 1.482(BPD) + 0.1676(BPD)^2$	1.36	1.27
HC	$GA = 8.96 + 0.540(HC) + 0.0003(HC)^3$	1.23	1.16
AC	$GA = 8.14 + 0.753(AC) = 0.0036(AC)^2$	1.31	1.24
FL	$GA = 10.35 + 2.460(FL) + 0.170(FL)^2$	1.28	0.92
BPD, AC	$GA = 9.57 + 0.524(AC) + 0.1220(BPD)^2$	1.18	1.10
BPD, HC	$GA = 10.32 + 0.009(HC)^2 = 1.3200(BPD) + 0.00012(HC)^3$	1.21	1.10
BPD, FL	$GA = 10.50 + 0.197(BPD)\,(FL) = 0.9500(FL) = 0.7300(BPD)$	1.10	0.89
HC, AC	$GA = 10.31 + 0.012(HC)^2 + 0.3850(AC)$	1.15	1.04
HC, FL	$GA = 11.19 + 0.070(HC)\,(FL) + 0.2630(HC)$	1.04	0.86
AC, FL	$GA = 10.47 + 0.442(AC) + 0.3140(FL)^2 - 0.0121(FL)^3$	1.11	0.90
BPD, AC, FL	$GA = 10.61 + 0.175(BDP)\,(FL) + 0.2970(AC) + 0.7100(FL)$	1.06	0.88
HC, AC, FL	$GA = 11.38 + 0.070(HC)\,(FL) + 0.9800(BPD)$	1.04	0.86
HC, AC, FL	$GA = 10.33 + 0.031(HC)\,(FL) + 0.3610(HC) + 0.0298(AC)\,(FL)$	1.03	0.85
HC, AC, BPD	$GA = 10.58 + 0.005(HC)^2 + 0.3635(AC) + 0.02864(BPD)\,(AC)$	1.14	1.09
BPD, HC, AC, FL	$GA = 10.85 + 0.060(HC)\,(FL) + 0.6700(BPD) + 0.1680(AC)$	1.02	0.86

BPD, Biparietal diameter; *GA,* gestational age; *HC,* head circumference; *AC,* abdominal circumference; *FL,* femur length.

burgh, and fulfilled the following criteria: (1) regular menses before conception, (2) an accurate date of the last menstrual period, (3) a positive pregnancy test result by 7 weeks' gestation, and (4) a pelvic examination to assess uterine size by 8 weeks' gestation. Patients with diseases known to affect fetal growth (e.g., diabetes mellitus) were excluded. Patients with multiple gestations were not accepted into the study group. The gestational ages of the study population ranged from 13 to 43 weeks. All the neonates weighed between the 10th and 90th percentiles at delivery.[10]

Each ultrasonographic examination was performed with commercially available real-time linear array ultrasonography equipment with either a 3.5 or 5.0 MHz transducer (General Electric, RT 3000 and RT 3200). Standard techniques were used for measurement of biparietal diameter,[6] head circumference,[11] abdominal circumference,[2] femur length,[3] and radius.[9] Electronic calipers set at a speed of sound in tissue of 1540 m/sec were used for linear measurements. The abdominal and head circumference were obtained directly from the ultrasonography unit after a tracing was made around the image on the screen with a track ball.

The robustness of the regression methods used by Hadlock et al.[8] to analyze their Houston data was assessed in two ways. First, the estimated parameters from the Houston equations were applied to our Pittsburgh data to generate predicted gestational ages. These predicted values were then compared with the actual gestational ages to assess an error of prediction, defined as the standard deviation of the residual values. Second, the functional forms of the Houston equations were used to reestimate the regressions with Pittsburgh data. The standard deviations of the resulting regression equations were then compared with those reported from Houston.

The assessment of whether radius length improves the prediction of gestational age was determined by running regressions with and without radius length. The statistical significance of regression coefficients for radius length was taken to reflect an independent, incrementally important, predictive role. Improvement in the coefficient of determination (R^2) and reduction in the standard deviation of the regression were also taken to reflect improvements in prediction.

Results

The performance of the regression equations estimated from Houston data as applied to the Pittsburgh population is shown in Table I. The standard deviations of the regressions for Pittsburgh data were calculated from the difference between predicted and actual gestational ages. Interestingly, predicted gestational ages showed less variable divergence from actual values for the Pittsburgh data than for the Houston data from which the prediction equations were obtained. This may be due to a more homogeneous patient population at Magee-Womens Hospital or to consistency in ultrasonographic measurements among a small staff of experienced ultrasonographers.

When the structure of the Houston equations were used to reestimate the regressions with Pittsburgh data, the Houston equations performed quite well (Table II). The coefficients and standard deviation of the regressions can be compared with those of Table I. In general, these comparisons show the figures to be quite similar.

The addition of several fetal measurements generally improves their predictive accuracy. This can be seen

Table II. Reestimation of Houston equations with Pittsburgh data

Equation	Without radius		With radius	
	SD (wk)	R^2	*SD (wk)*	R^2
One variable				
GA = 6.99 + 2.25(BPD) + 0.104(BPD)^2	1.26	96.3	1.04	97.5
GA = 7.10 + 0.661(HC) + 00020(HC)^3	1.11	97.1	0.96	97.9
GA = 6.34 + 0.916(AC) − 0.00044(AC)^2	1.22	96.5	1.02	97.6
GA = 9.37 + 2.97(FL) + 0.115(FL)^2	0.91	98.1	0.89	98.2
Two variables				
GA = 9.13 + 0.55(AC) = 0.114(BPD)^2	1.10	97.2	0.96	97.9
GA = 9.62 + 1.20(BPD) +0.014(HC)^2 − 0.116(HC)^3	1.09	97.2	0.95	97.9
GA = 9.18 + 2.14(FL) + 0.427(BPD) + 0.124(BPD) (FL)	0.85	98.3	0.84	98.4
GA = 10.09 + 0.388(AC) + 0.012(HC)^2	1.03	97.5	0.92	98.0
GA = 10.56 + 0.304(HC) + 0.068(HC) (FL)	0.85	98.3	0.81	98.5
GA = 9.11 + 0.689(AC) + 0.014(FL)^3	1.09	97.2	0.91	98.1
Three variables				
GA = 9.29 + 1.98(FL) + 0.173(AC) + 0.114(BPD) (FL)	0.83	98.4	0.82	98.5
GA = 11.18 + 0.96(BPD) + 0.072(HC) (FL)	0.86	98.3	0.81	98.5
GA = 8.52 + 0.514(HC) + 0.047(AC) (FL)	0.86	98.3	0.82	98.4
GA = 9.99 + 0.398(AC) + 0.013(HC)^2 − 0.0015(BPD) (AC)	1.04	97.5	0.91	98.1
Four variables				
GA = 10.65 + 0.139(AC) + 0.739(BPD) + 0.63(HC) (FL)	0.85	98.3	0.80	98.5

GA, Gestational age; *BPD,* biparietal diameter; *HC,* head circumference; *AC,* abdominal circumference; *FL,* femur length.

from Table II. For example, the importance of femur length can be inferred by comparing equations 7, 9, and 10 with the corresponding equations 1, 2, and 3. The standard deviation of the regressions with femur length are lower than those without femur length. Of particular note is the fact that the standard deviation of the regressions cannot be improved on by adding variables beyond the two-variable case. That is, the standard deviations for biparietal diameter and femur length, as well as head circumference and femur length, are as low as that obtained when four variables (biparietal diameter, femur length, head circumference, abdominal circumference) are used.

The usefulness of radius length as a predictor of gestational age was assessed by adding radius to each of the regression models shown in Table II. In almost all cases radius provides an independent, statistically significant contribution to the prediction of gestational age beyond that of the variables already in the equation. The standard deviations of the regression are lower and the R^2 values are higher.

To assess how radius compares with other fetal measurements, the best regression equation for each fetal measurement or combination of fetal measurements was estimated, as shown in Table III. The criteria for "best regression" was the same as that used by Hadlock et al.[8]—the lowest standard deviation and highest R^2 associated with the equation in which all variables were significant. Radius did not significantly improve an equation when another long bone, femur length, was included. Radius length, however, improved prediction when fetal measurements other than the femur length (i.e., head circumference, biparietal diameter, abdominal circumference) were used.

Comment

Sabbagha et al.[12] studied the growth rate of the biparietal diameter in 67 pregnant monkeys with known breeding dates. He was able to divide his study group into four percentile divisions: 10th to 24th, 25th to 49th, 50th to 74th, and 75th to 90th. Once a monkey was placed into one of these categories, his subsequent growth would remain within the confines of that percentile range. This was particularly true for the lower (probability 0.92%) and upper (probability 0.93%) end scale. A similar growth pattern exists for the biparietal diameter in the human.[13] Other fetal parameters, however, may not vary in the same direction (e.g., a fetus with a 75th percentile biparietal diameter and 25th percentile abdominal circumference[14]). As a result, the dependence on a single fetal measurement to assess gestational age in the third trimester has a 95% confidence interval of ±3 weeks or more. By combining the gestational age estimates of fetal body parts with varying growth rates (and differential susceptibilities to growth inhibition), Hadlock et al.[7, 8] were able to significantly reduce the confidence interval.

Our data confirm certain aspects of the studies performed by Hadlock et al.[7, 8] while refuting others. Table IV compares the correlation coefficients for the present investigation with Hadlock et al.[8] The femur length was found to be superior to the BPD in both studies as a single measurement of gestational age. Yagel et al.[15] came to a similar conclusion.

Table III. Variability and coefficients of determination for best Pittsburgh regression equations

Standard fetal measurements	*SD (wk)*	R^2	*Standard fetal measurements*	*SD (wk)*	R^2
FL	0.91	98.1	HC, BPD, FL	0.79	0.985
HC	1.11	96.9	HC, AC, FL	0.80	0.985
AC	1.22	96.5	HC, FL, RAD	0.80	0.985
BPD	1.26	96.3	BPD, FL, RAD	0.84	0.984
RAD	1.33	95.9	AC, FL, RAD	0.85	0.983
FL, HC	0.80	98.5	HC, AC, RAD	0.90	0.981
FL, BPD	0.85	98.3	BPD, HC, RAD	0.92	0.980
FL, AC	0.86	98.2	BPD, AC, RAD	0.94	0.979
RAD, FL	0.89	98.2	BPD, AC, FL	0.98	0.984
RAD, HC	0.93	98.0	BPD, AC, HC	1.02	0.976
RAD, BPD	1.01	97.6	BPD, HC, AC, FL	0.80	0.985
RAD, AC	1.01	97.6	BPD, HC, FL, RAD	0.80	0.985
HC, AC	1.02	97.6	AC, HC, FL, RAD	0.80	0.985
HC, BPD	1.09	97.2	AC, BPD, FL, RAD	0.82	0.984
			AC, HC, BPD, RAD	0.89	0.981
			BPD, HC, FL, AC, RAD	0.80	0.985

FL, Femur length; *HC,* head circumference; *BPD,* biparietal diameter; *AC,* abdominal circumference; *RAD,* radius length.

Table IV. Correlation coefficients of various fetal measurements versus gestational age

Fetal measurements	*Hadlock et al.*[8]	*Current study*
Biparietal diameter	96.7	96.3
Head circumference	97.3	97.1
Abdominal circumference	96.9	96.5
Femur length	98.0	98.1
Femur length, head circumference	98.0	98.5
Head circumference, abdominal circumference, femur length	98.1	98.3
Biparietal diameter, head circumference, abdominal circumference, femur length	98.1	98.3

The study of Hadlock et al.[8] and the current investigation show a slight increase in R^2 when two, rather than one, fetal parameters are used to evaluate gestational age. The combination of head circumference and femur length resulted in the best coefficient of determination with the least variability. The data in Table III indicate that the addition of other fetal biometric data (biparietal diameter, abdominal circumference, and radius) did not significantly improve the results obtained from the combination of the head circumference and femur length. Although Hadlock et al.[8] state that there is a significant reduction in variability when four parameters rather than one are used to assess gestational age, the data they provide also indicate that the variability and correlation coefficient for head circumference and femur length are comparable to those of the combination of biparietal diameter, head circumference, abdominal circumference, and femur length.

It should be emphasized that neither our data nor the data of Hadlock et al.[8] found a point of diminishing returns by adding up to five fetal parameters in the determination of gestational age, i.e., the error inherent in measuring several distinctly different structures did not significantly affect variability. We therefore concur with Hadlock et al.[8] that a standard ultrasonographic examination should include measurements of the biparietal diameter, head circumference, abdominal circumference, and at least one long bone. By completing a fetal growth profile rather than obtaining one or two selected measurements, the ultrasonographer will be in a better position to detect not only abnormalities of fetal growth but also certain congenital anomalies.

Hadlock et al.[16] have previously shown that regression equations developed from a white population were applicable to fetuses from a population with different socioeconomic and racial characteristics. Our study confirms their findings. Other institutions performing obstetric ultrasonography should not assume that they would obtain comparable results. An institutionally derived regression equation will reduce the variability in the estimation of gestational age while only marginally altering the coefficient of determination. We would, therefore, suggest that each obstetric ultrasonography service evaluate several hundred patients in a manner similar to ours. If the variability in the assessment of gestational age is significantly affected, we would recommend that an institutional, rather than a published, formula be used.

We had hoped that the radius length would further narrow the variability inherent in the ultrasonographic assessment of gestational age for two reasons. First, when the fetal long bones are compared, the growth rates of the femur and radius are the most disparate.[17] Second, with advancing gestation the right femur be-

comes significantly longer than the left, whereas the length of the radius is the same on either side.[17] Thus the varying slopes of the regression equation for femur length and radius length versus gestational age and the increased variability obtained in randomly measuring either the left or the right femur implied to us that radius length would either provide additional information or replace femur length as the long bone measurement of choice. Unfortunately, as outlined on Table III, radius length had the largest variability and poorest correlation coefficient of the five fetal parameters we evaluated. When the radius length was combined with other fetal biometric data it did not significantly improve any of the equations that included femur length. These findings indicate that the theoretic difference between radius length and femur length mentioned previously is not clinically significant.

In conclusion, we have shown that several measurements of fetal size (femur length, head circumference, biparietal diameter, abdominal circumference, and radius length) have a high correlation with gestational age when considered individually. The combination of more than one fetal measurement results in only a slightly higher estimated correlation with gestational age but substantially reduces the error of estimate. For our normal patient population, the combination of head circumference and femur length was the most efficacious. The addition of other fetal parameters did not significantly improve the estimate of gestational age. Finally, we have confirmed the generalizability of carefully obtained fetal biometric data across population groups.

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Phase II trial of ifosfamide and mesna in leiomyosarcoma of the uterus: A Gynecologic Oncology Group study

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OBJECTIVE: The purpose of this study was to evaluate the activity of ifosfamide (isophosphamide) in patients with advanced or recurrent leiomyosarcoma not previously exposed to chemotherapy.

STUDY DESIGN: This is a phase II groupwide study of the Gynecologic Oncology Group. Thirty-five patients were treated with ifosfamide 1.5 gm/m^2 daily intravenously for 5 days with mesna (mercaptoethane sodium sulfonate). Fifty-six patients had received prior abdominal hysterectomy and 15 prior radiotherapy. The dose was reduced to 1.2 gm/m^2 daily in patients who had received prior radiotherapy.

RESULTS: Gynecologic Oncology Group grade III or IV granulocytopenia occurred in 4 (11%) patients, and none had grade IV thrombocytopenia. One (2.8%) patient had grade IV neurotoxicity. Partial responses were observed in 6 of 35 (17.2%) patients. The 95% confidence interval for response was 6.6% to 33.7%.

CONCLUSION: These results indicate that ifosfamide has modest activity in patients with advanced or recurrent leiomyosarcomas of the uterus. (Am J Obstet Gynecol 1992;166:556-9.)

Key words: Ifosfamide, mesna, leiomyosarcoma of the uterus

Ifosfamide is an alkylating agent structurally similar to cyclophosphamide, which is highly active in the treatment of a number of nongynecologic and gynecologic neoplasms, including lymphomas[1] and testicular[2] and ovarian carcinomas.[3]

Like doxorubicin, ifosfamide possesses activity in the treatment of soft tissue sarcomas. Bramwell et al.[4] compared cyclophosphamide with ifosfamide in advanced soft tissue sarcomas. The response rate in previously untreated patients was 25% with ifosfamide and 13% with cyclophosphamide. Other authors[5, 6] have confirmed response rates of 23% to 65% in previously treated patients with soft tissue sarcomas.

Because of these promising results, the Gynecologic Oncology Group initiated a phase II trial of ifosfamide and mesna in patients with uterine sarcomas refractory to surgery and radiotherapy but untreated with previous chemotherapeutic agents. In mixed müllerian tumors of the uterus, ifosfamide and mesna produced an overall response rate of 32.2% and a complete response rate of 17.9% in 28 patients evaluable for response.[7] The current report includes patients with leiomyosarcomas of the uterus who have had no prior chemotherapy.

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Material and methods

Patients with histologically proved advanced or recurrent leiomyosarcoma of the uterus not amenable to curative therapy but unexposed to prior chemotherapeutic agents were eligible for this study. Additional eligibility criteria included measurable disease that could be defined in at least two dimensions by physical examination or medical imaging techniques. Ascites and pleural effusions were not considered measurable disease. Also required were Gynecologic Oncology Group performance status 2 or better[8]; an interval of ≥3 weeks since any prior tumor-oriented therapy; absence of significant infection; recovery from recent surgery or radiotherapy; and adequate hematologic white blood cell count ≥3000/μl, platelet count ≥100,000/μl), hepatic (serum bilirubin, alanine transferase, and alkaline phosphatase two × normal or less), and renal (serum creatinine ≤2.0 mg/dl or creatinine clearance ≥50 ml/min) function tests. Informed consent was obtained from all subjects, and none was treated until the study was approved by the Human Investigations Committee of the participating institution.

Criteria for exclusion were absence of measurable disease, prior chemotherapy, Gynecologic Oncology Group performance status of ≥3, presence of a second

Table I. Patient characteristics and response to ifosfamide

Patient characteristic	*Partial response*	*Stable disease*	*Increasing disease*	*Total*
No. of evaluable patients	—	—	—	35
Age (yr, median and range)	—	—	—	53 (32-65)
Gynecologic Oncology Study performance status				
0	4	3	7	14
1	1	6	6	13
2	1	1	6	8
Prior radiotherapy				
Yes	4	3	8	15
No	2	7	11	20
Prior hysterectomy				
Yes	5	10	11	26
No	1	0	8	9

malignancy excluding squamous or basal skin lesions, or the presence of microhematuria in excess of the institutional lowest normal range.

Ifosfamide was administered intravenously at a dose of 1.5 gm/m² daily for 5 days every 4 weeks. This dose was reduced to 1.2 gm/m² in patients with prior radiotherapy. Mesna was given intravenously, 300 mg/m², every 4 hours for three doses after each dose of ifosfamide. History and physical examination with assessment of evaluable lesions, Gynecologic Oncology Group performance status, serum chemistry results, blood urea nitrogen, creatinine, and microscopic urinalysis were performed before each cycle of therapy, and complete blood cell count and differential and platelet counts were obtained weekly. Chest radiograph was required on entrance to study and every 3 weeks if a marker lesion was present. Abdominal pelvic computed tomographic scans were used unless tumor parameters were assessed by examination or chest radiograph.

Drug administration was delayed after hematologic toxicity until white blood cell count was >3000 μl, platelet count >100,000/μl, and granulocyte count >1500/μl. Patients were considered evaluable for toxicity if just one course of therapy was given and evaluable for response after a single course of therapy if there was clear-cut progression. In other cases patients remained on study until disease progression was noted or adverse effects prevented further therapy. Response duration was calculated from the start of therapy until documentation of disease progression. Patients were considered to have stable disease if there was no progression of disease during therapy for 4 months.

Results

Between May 1986 and June 1989, 36 patients were entered into the study. One patient refused therapy before the first administration, leaving 35 patients evaluable for response and toxicity. The median age of evaluable patients was 53 years (range 32 to 65 years). Twenty-six patients had undergone prior hysterectomy, two had laparotomy only, and seven had had no surgery. Fifteen patients (42.9%) had received prior radiotherapy. Fourteen patients had Gynecologic Oncology Group performance status 0, 13 had Gynecologic Oncology Group performance status 1, and eight had Gynecologic Oncology Group performance status 2.

Response. Table I outlines patient characteristics and the clinical results of therapy with ifosfamide. Six patients (17.2%) had partial responses to therapy, and 10 patients (28.6%) had stable disease. Response duration was 2.9 to 12.4 months, with a median response duration of 3.8 months. Response by Gynecologic Oncology Group performance status is shown in Table I. Median age for responders was 52 years (range 38 to 62 years). Four responses occurred in patients receiving prior radiotherapy; the other two responders had not received radiotherapy. Responders received a median 5.0 courses of chemotherapy (range three to seven courses). Responses by adverse hematologic effects are also detailed in Table I. Median survival is 6.0 months.

Toxicity. Nineteen patients were treated at a dose of 1.5 gm/m²/day for 5 days; the remainder had therapy initiated at the 1.2 gm/m² dose because of prior radiotherapy. Twelve patients (34.4%) experienced Gynecologic Oncology Group grade 3 or 4 leukocytopenia (white blood cell count ≤200/μl), and four (11.4%) had granulocyte counts <1000/μl. The median white blood cell count nadir for all patients with leukocytopenia was 1900/μl (range 600/μl to 3400/μl). Adverse effects are outlined in Table I. No patients developed significant thrombocytopenia. No patient experienced granulocytopenic fever or thrombocytopenic hemorrhage.

Other adverse effects are listed in Table II. Grade 4 neurotoxicity developed in one patient. This patient became lethargic and disoriented but responded to the withdrawal of ifosfamide and mesna. Hematuria was not observed in this patient population in spite of prior

Table II. Other adverse effects

Adverse effect	Grade 0	1	2	3	4	Total
Thrombocytopenia	34	1	0	0	0	35
Granulocytopenia	30	0	1	0	4	35
Gastrointestinal	25	2	7	1	0	35
Dermatologic	26	1	3	5	0	35
Renal	32	3	0	0	0	35
Cardiac	34	1	0	0	0	35
Neurologic (central)	33	1	0	0	1	35
Pulmonary	34	0	1	0	0	35
Fever	34	1	0	0	0	35
Anemia	34	0	1	0	0	35

radiotherapy in many subjects. There was no renal toxicity exceeding grade 1 (three patients).

Comment

Uterine sarcomas continue to present a challenge to the gynecologic oncologist. Patients with malignancy confined to the uterus and cervix have a 5-year survival of 50% at best,[9] and those with stage III and IV disease rarely survive for this length of time. Although the use of postoperative pelvic radiotherapy may reduce the risk of pelvic recurrence, this method of treatment has not changed recurrence rates or survival. Furthermore, adjunctive chemotherapy with doxorubicin does not affect survival.[10]

A variety of drugs have been used in the treatment of advanced or metastatic uterine sarcomas. Few early reports separate leiomyosarcomas from other more common types, such as mixed müllerian tumors. Omura et al.[11] compared doxorubicin with or without dacarbazine in 146 evaluable patients with measurable uterine sarcomas. When treated with doxorubicin alone, only four of 41 patients (9.8%) with mixed müllerian tumors responded to therapy. In contrast, seven of 28 patients (25.0%) with leiomyosarcoma responded to doxorubicin. With the addition of dacarbazine, response rates were 22.6% and 30%, respectively. This was the first report suggesting a differential sensitivity among uterine sarcomas to chemotherapy. In a later study, cisplatin produced responses in five of 28 patients (18%) with mixed müllerian tumors refractory to prior doxorubicin.[12] In contrast, only one of 19 patients (5.3%) with refractory leiomyosarcoma responded to cisplatin.[13] Responses to cisplatin in previously untreated patients were also rare. Because of these findings, the Gynecologic Oncology Group has evaluated new agents for the treatment of uterine sarcomas in patients grouped by histologic type.

In a phase II study by the Gynecologic Oncology Group,[7] 32.2% of 28 evaluable patients with mixed müllerian tumors that had not been exposed to chemotherapy responded to ifosfamide therapy. Five patients (17.9%) had complete responses to therapy. These findings suggest that ifosfamide is a drug with single-agent activity in mixed müllerian tumors. Although only 17.2% of leiomyosarcomas were sensitive to ifosfamide in the current report, this degree of activity is comparable to that of doxorubicin as a single agent. These findings suggest that ifosfamide and doxorubicin with or without dacarbazine would be valuable combinations in the treatment of advanced uterine leiomyosarcomas. Furthermore, if response rates >50% or 60% can be attained, such a combination might be considered in the adjuvant therapy of completely resected uterine leiomyosarcomas.

Ifosfamide-based combination regimens are showing promise in the management of soft tissue sarcomas. This drug has been combined effectively with doxorubicin in phase II studies. Schutte et al.[14] reported a 36% response rate, including 6.3% complete responses, in 126 evaluable patients treated with a 1-day regimen of ifosfamide and doxorubicin. Sledge et al.[15] reported 16 of 39 (41%) responders with a similar regimen. Elias et al.[15] added dacarbazine to these two drugs and observed responses in 13 of 29 (45%) patients with three or 10% complete responses. Combinations including ifosfamide in the treatment of uterine leiomyosarcoma will be studied in future Gynecologic Oncology Group trials.

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ics (CA 19502), Indiana University Medical Center (CA 21720), Bowman Gray School of Medicine of Wake Forest University (CA 21946), The Albany Medical College of Union University (CA 27469), University of California Medical Center at Irvine (CA 23765), Illinois Cancer Council (CA 27806), University of Pittsburgh School of Medicine (unfunded), St. Louis University Medical Center (CA 35571), Stanford University Medical Center (unfunded), Eastern Virginia Medical School (CA 40296), and State University of New York at Stony Brook (unfunded).

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Prenatal diagnosis of autosomal recessive polycystic kidney disease: Variable outcome within one family

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Autosomal recessive polycystic kidney disease is frequently diagnosed in utero by obstetric ultrasonography. We report a case in which there were varying outcomes of this disorder in three affected fetuses in a family. Recognition of variable expression within one family is important when parents are considering termination of a pregnancy with an affected fetus. (AM J OBSTET GYNECOL 1992;166:560-7.)

Key words: Fetus, abnormalities; fetus, genitourinary tract; fetus, ultrasound studies

Autosomal recessive polycystic kidney disease has an incidence of approximately 1 in 40,000. It is frequently diagnosed in utero by obstetric ultrasonography or can be seen in the newborn with bilateral flank masses, renal failure, or oligohydramnios-related pulmonary hypoplasia. The kidneys are symmetrically enlarged and characterized microscopically by severe cystic dilatation of the renal collecting tubules and ducts. Hepatic fibrosis is always present, and its severity tends to be inversely proportional to the degree of renal involvement. Renal disease dominates the clinical picture in the perinatal period and is often associated with renal failure and early death. Liver disease dominates the presentation in older children, and renal manifestations are frequently mild in this subgroup. We describe a case in which there were varying outcomes in three affected fetuses from one family.

Case report

A 28-year-old woman, gravida 3, para 3, conceived three children affected with the perinatal form of autosomal recessive polycystic kidney disease. Two children died soon after birth, and one is still alive at age 3½ years. Prenatal ultrasonography diagnosed severe disease in two of the siblings, including the one long-term survivor.

This couple's first pregnancy resulted in a female infant at 38 weeks' gestation who died of severe pulmonary hypoplasia at 8 hours. Autopsy results were typical of autosomal recessive polycystic kidney disease. Because of a 25% recurrence risk, the family's second pregnancy was monitored ultrasonographically. Ultrasonography performed at 16.5 and 19.5 weeks' gestation was normal. The family was counseled regarding the variable expression of the disease and the possibility that it could manifest later in pregnancy or childhood.

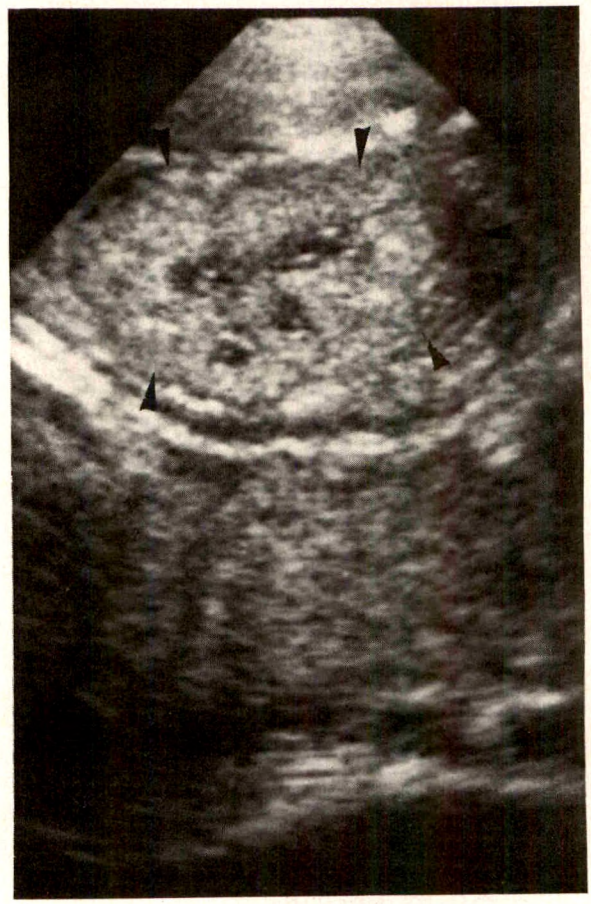


Fig. 1A. Autosomal recessive polycystic kidney disease at 35 weeks' gestation (second affected fetus). Sagittal scan of fetal kidney *(arrowheads)* shows severe enlargement with diffuse increase in echogenicity.

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Fig. 1B. Autosomal recessive polycystic kidney disease (second affected fetus). Microscopic section of kidneys shows normal glomeruli with severe cystic dilatation of renal tubules *(arrowheads)*.

The parents elected to carry the pregnancy to completion. Ultrasonography at 35 weeks showed severe oligohydramnios, enlarged fetal kidneys (Fig. 1A), and a small bladder. This pregnancy resulted in spontaneous vaginal delivery at 37 weeks of a male infant who died of severe pulmonary hypoplasia at 28 hours. Autopsy results were diagnostic of autosomal recessive polycystic kidney disease (Fig. 1B). The family conceived a third time and again underwent ultrasonographic monitoring. Ultrasonography at 20.5 weeks showed enlarged and echogenic kidneys diagnostic for this disease. Amniotic fluid was normal and a small fetal bladder was identified. In spite of early fetal diagnosis and the concern for a fatal outcome, the parents elected to continue the pregnancy. A follow-up sonogram at 30 weeks' gestation showed severe oligohydramnios and enlarged echogenic kidneys with a peripheral hypoechoic rim typical of autosomal recessive polycystic kidney disease (Fig. 1C). This pregnancy culminated with a spontaneous vaginal delivery at 37.5 weeks. Postnatal ultrasonography showed enlarged and echogenic kidneys and a normal liver. The child is currently 3½ years old with medically treated hypertension and normal renal function.

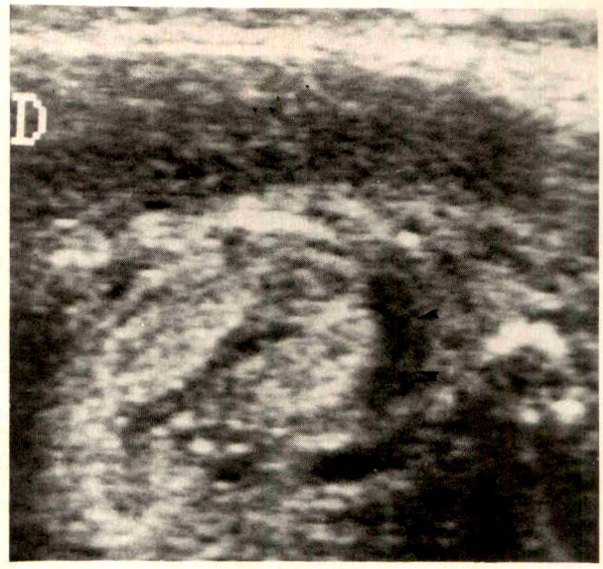


Fig. 1C. Autosomal recessive polycystic kidney disease, 30 weeks' gestation (third affected fetus). Sagittal scan of kidney shows severe enlargement, increased echogenicity, and a peripheral hypoechoic rim *(arrowheads)*.

Comment

Prenatal or neonatal diagnosis of autosomal recessive polycystic kidney disease frequently has a fatal outcome because of renal failure or pulmonary hypoplasia. Previous reports have stated that severity of disease in affected newborns is consistent within a given family.[1] However, this case report supports the potential for prolonged survival, even when previous siblings have died of the severe perinatal form of autosomal recessive polycystic kidney disease. The literature on siblings is limited but supports the concept that variable expression reflects the natural history of the disease rather than the occurrence of separate genetic entities.[2]

When a pregnancy at risk is diagnosed as having a fetus with autosomal recessive polycystic kidney disease, the parents can be counseled with the option of termination; however, it should be noted that the outcome is not invariably fatal. Conversely, the absence of ultrasonographic manifestations on initial fetal evaluation does not exclude the possibility of delayed expression, either in utero or after birth.

Recognition of variable expression within one family is important for genetic counseling or when parents are considering termination of a pregnancy with an affected fetus.

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Laboratory and clinical comparison of a rapid versus a classic progesterone radioimmunoassay for use in determining abnormal and ectopic pregnancies

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A rapid, solid-phase, nonextraction progesterone kit (n = 36) was compared with an established extraction method, liquid phase tritium radioimmunoassay for progesterone (n = 79). Logistic regression of the rapid method over the extraction method gave a correlation coefficient of r = 0.968. Logistic regression gave a discriminatory zone (abnormal vs normal pregnancy) of 22.58 ng/ml by the rapid method compared with 15 ng/ml for the extraction method (log Y = 0.253 + 0.936 log X). Similar sensitivity (100% and 100%), specificity (63% and 59%), and predictive values of positive and negative tests (positive predictive value = 91% and 75%, negative predictive value = 100% and 100%) were found using a discriminatory level of 15 and 20 ng/ml for the extraction method (n = 79) and rapid method (n = 76), respectively. Clinically, curettage for patients with values less than these discriminatory zones would have given unacceptable rates for termination of a potentially normal pregnancy (38%). Clinical comparison of abnormal (ectopic or abortion) and normal (term pregnancy) outcomes in two populations of women presenting with first-trimester bleeding revealed that progesterone levels would accelerate the workup in only a small percentage (27%, 15 of 56) of patients with ectopic pregnancies. The rate of ruptured ectopic pregnancies in the observed patients was similar (20%, 3 of 15) to studies using progesterone levels prospectively. Therefore in this retrospective analysis progesterone levels would have given no greater benefit than conventional management. (Am J Obstet Gynecol 1992;166:562-6.)

Key words: Progesterone levels in abnormal pregnancies

Ectopic pregnancies have abnormal corpus luteal function not attributable to a decrease in bioactive human chorionic gonadotropin (hCG).[1, 2] Keko and others[3-5] have suggested that serum progesterone levels provide rapid differentiation between normal and abnormal pregnancies (ectopic, inevitable abortion). In those studies a rapid, nonextraction assay kit was used, and each laboratory established its own discriminatory zone. Normal pregnancies[1, 3, 4, 6-8] or "patients presenting to the emergency room identified by housestaff as being at risk for ectopic pregnancy,"[5, 9] were used as controls.

The purpose of our study was (1) to compare the precision of a nonextraction, solid-phase, iodine 125 radioimmunoassay (RIA) procedure to an extraction, liquid-phase, tritium RIA procedure, (2) to determine the discriminatory value of each assay necessary to differentiate a bleeding intrauterine pregnancy that progresses normally to a live birth from an abnormal pregnancy (ectopic or inevitable abortion), and (3) to retrospectively evaluate the clinical usefulness of serum progesterone measurement compared with routine management.

Material and methods

Patients. From October 1988 to February 1989 all patients seen in the emergency room at the University of Miami who were at >4 weeks and <12 weeks' gestation (from last menstrual period) with abdominal pain and abnormal uterine bleeding had a serum sample frozen at −20° C for later evaluation. Documentation of pregnancy by positive β-hCG, absence of ovulation-inducing drugs, serial β-hCG levels, ultrasonography, serial examinations, and a final diagnosis by pathology specimen was available for 79 patients. Patients not taken immediately for laparoscopy or laparotomy (positive culdocentesis) were followed with β-hCG titers every 48 hours. Transvaginal ultrasonography was obtained when titers failed to rise ≥66%, a mass was palpated, or marked abdominal tenderness was noted. If all pregnant patients seen with abnormal vaginal bleeding and abdominal tenderness in the emergency room at Jackson Memorial Hospital required ultrasonography, the demand potentially could exceed the supply level of that hospital. Thus the senior residents triaged

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patients according to signs and symptoms and requested a priority ultrasonography for "high-risk" patients.

In the study sample from the University of Miami, 42 ectopic pregnancies, 21 inevitable or spontaneous abortions, and 16 pregnancies with first trimester uterine bleeding subsequently carried to term with delivery of a normal infant were investigated. Serum progesterone levels were performed in these samples by ether extraction with a recovery >90% and liquid-phase, ^{3}H RIAs.[10] Sensitivity, specificity, and predictive indices were determined.

In 36 cases (10 randomly selected ectopic pregnancies, 10 randomly selected inevitable or spontaneous abortions, and 16 intrauterine pregnancies carried to term), adequate serum was available in the samples to perform duplicate, nonextraction, solid-phase, ^{125}I RIAs (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, Calif.). The manufacturer's methodology with a 3-hour incubation was used. The extraction method and rapid method were compared by methods described by Rodbard.[11] Cross-reactivity with 17-hydroxyprogesterone in the classic extraction method ^{3}H progesterone RIA was 6.7% and 0.3% in the nonextraction rapid progesterone kit.

Additionally, from January 1990 to November 1991, 40 patients were studied with the same criteria at the University of Utah Hospital. In this study 14 ectopic pregnancies, 8 inevitable or spontaneous abortions, and 18 pregnancies with first trimester uterine bleeding, which subsequently carried to term delivery, were evaluated by the rapid method. Duplicate, nonextraction, solid-phase, ^{125}I RIAs (Coat-A Count, Diagnostic Products Corporation, Los Angeles, Calif.) were performed on these patients. The sensitivity, specificity, and predictive values were determined on the combined group (n = 76) from both institutions and compared with the patients evaluated by the extraction method (n = 79).

Results

Fig. 1 shows the serum progesterone levels for patients with ectopic pregnancies (n = 42), inevitable abortions (n = 21), and normal intrauterine pregnancies carried to term (n = 16), with the extraction, liquid-phase, tritium RIA method (extraction method). The intraassay and interassay coefficients of variation were 7.5% and 9.5%, respectively. The sensitivity and specificity of a level <15 ng/ml in detecting an abnormal pregnancy was 100% (63/63) and 63% (10/16), respectively; hence, the predictive value of a positive test (<15 ng/ml = abnormal pregnancy) was 91.3% (63/69) and of a negative test (>15 ng/ml – normal pregnancy), 100% (10/10).

Fig. 1 also shows the serum progesterone in 76 patients evaluated by the rapid, nonextraction, solid-phase, ^{125}I RIA method (rapid method). The intraassay and interassay coefficients of variation were 11% and 12%, respectively. The rapid kit using a discriminatory zone of <20 ng/ml to detect an abnormal pregnancy (n = 76; University of Miami, n = 36; University of Utah, n = 40) had a sensitivity of 100% (45/45), a specificity of 59% (20/34), and positive and negative predictive indices of 75% (42/56) and 100% (20/20), respectively.

The coefficient of variation between the rapid method and the extraction method at levels >20 ng/ml (by rapid method) was 13.8% and at levels <20 ng/ml, 20.2%. Logistic regression of the rapid method over the extraction method gave a correlation coefficient of r = 0.968 (n = 36). With Rodbard's methodology, the equation $\log Y = 2.53 + 0.936 \log X$ or $Y = 1.79X^{0.936}$ was derived.[11] This gave a discriminatory zone (abnormal vs normal pregnancy) of 22.58 ng/ml by the rapid method compared with 15 ng/ml for the extraction method. Using 22.58 ng/ml as the discriminatory zone gave the same sensitivity, specificity, and predictive values as 20 ng/ml in the rapid assay.

The clinical presentation of the patients studied from the University of Miami and the University of Utah prompted an immediate laparoscopic evaluation in 73% (41/56) of the ectopic pregnancies. In 27% (15/56) a rapid serum progesterone within 3 hours would have prompted an ultrasonographic evaluation before 48 hours when the second quantitative β-hCG was obtained. In 80% (12/15), observation was continued after the 48-hour β-hCG titer result because of equivocal findings noted by ultrasonography. In 20% (3/15) actual ultrasonography findings prompted laparoscopy. If low progesterone levels in association with no intrauterine pregnancy by transvaginal ultrasonography with a β-hCG ≥2500 mIU/ml were used to decide on immediate intervention by laparoscopy, 27% (4/15) of patients observed might have proceeded to earlier laparoscopy.

If extraction method progesterone levels (≤15 ng/ml) were used to indicate immediate intervention, including curettage, 38% (6/16) of intrauterine pregnancies would have been terminated. If the rapid progesterone level (<20 ng/ml) were used to prompt curettage, 38% (7/18) of intrauterine pregnancies would have been terminated. All intrauterine pregnancies had progesterone levels >9.8 ng/ml by the extraction method and 11.5 ng/ml by the rapid method.

In patients who underwent laparoscopy or laparotomy on presentation, 61% (25/41) had ruptured ectopic pregnancies and 39% (16/41) had unruptured ectopic pregnancies. In the group observed >72 hours before operative intervention, 20% (3/15) were ruptured and 80% (12/15) were unruptured.

A total of 48% of the abortions (14/29) were diagnosed within 48 hours by clinical features and ultra-

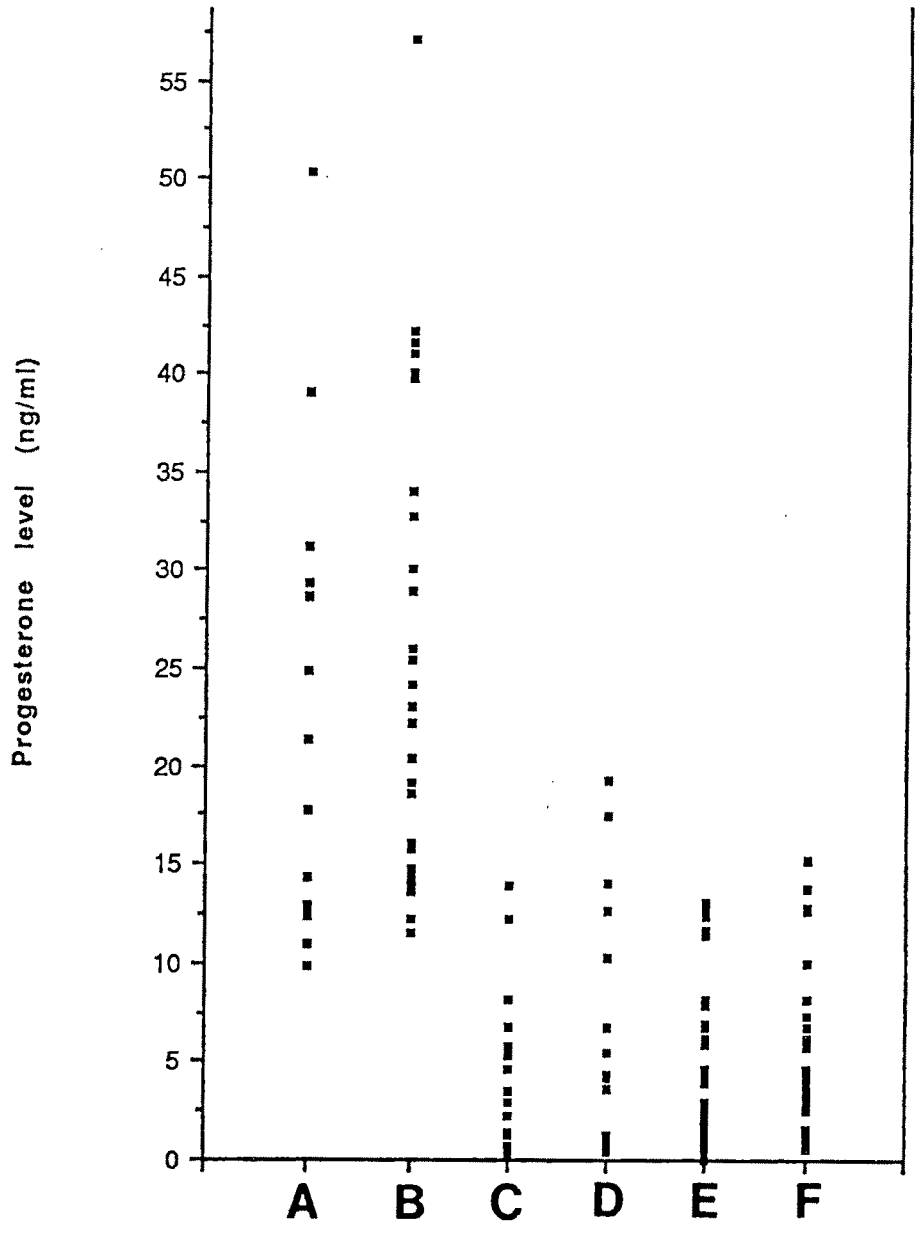


Fig. 1. Serum progesterone levels in patients presenting with first trimester bleeding and abdominal pain. *A*, Progesterone levels by extraction method in pregnancies carried to term. *B*, Progesterone levels by rapid method in pregnancies carried to term. *C*, Progesterone levels by extraction method in spontaneous or inevitable abortions. *D*, Progesterone levels by rapid method in spontaneous or inevitable abortions. *E*, Progesterone levels by extraction method in ectopic pregnancies. *F*, Progesterone levels by rapid method in ectopic pregnancies.

sonography. The remaining 52% (15/29) were observed. Of these, 33% (5/15) underwent laparoscopy and curettage.

Comment

This study demonstrates the need to evaluate available rapid progesterone kits compared with a published methodology to define appropriate discriminatory levels in each clinical setting before use. The variation rates in the two methods noted by clinical outcome (predictive values of positive and negative tests) prompted an elevation of the discriminatory value from 15 ng/ml to 20 ng/ml using the rapid progesterone kit. With this elevation in the discriminatory zone, a similar

predictive value of a positive test (<20 ng/ml) of 75% (vs 91% for extraction) and of a negative test (>20 ng/ml) of 100% (vs 100% for extraction) was obtained for the rapid progesterone kit. Logistic regression indicated the discriminatory zone should be 22.58 ng/ml for the rapid progesterone kit. Thus clinicians deciding to use progesterone levels must define in their laboratories appropriate discriminatory zones on the basis of the methodology used. No intrauterine pregnancy had a progesterone level <9.8 ng/ml by either method. While this study gave an acceptable predictive index of a negative test using a value >20 ng/ml as the discriminatory zone, we can envision that higher levels of progesterone may be found in such conditions as cornual ectopics.

It has been suggested that the incidence of rupture of ectopic pregnancies may be reduced with the use of a rapid progesterone and a transvaginal ultrasonography. In our experience, which did not use the serum progesterone prospectively, the incidence of ruptured ectopic pregnancy in the group observed longer than 72 hours (20%, 3/15) was similar to other reports using a serum progesterone in the work-up.[7] Additionally, in a majority of cases (80%, 12/15) ultrasonography was not diagnostic for an ectopic, and observation was continued. However, in 20% (3/15), ultrasonography prompted immediate laparoscopy. If a low progesterone level combined with an hCG level ≥2500 without an intrauterine pregnancy by ultrasonography was used to prompt laparoscopy, 27% (4/15) would have been evaluated sooner. The fact that rupture of ectopic pregnancies occurred during the work-up emphasizes the need for a high degree of suspicion throughout the evaluation. Progesterone levels have been thought to expedite the diagnosis through further studies (i.e., ultrasonography or laparoscopy); however, in this study ultrasonography was nondiagnostic in 80% and the decision to evaluate by laparoscopy again fell to the practitioner. In this series, it is not clear that a benefit greater than our routine evaluation would result from the use of a progesterone level as part of the work-up.[7] The discriminatory values chosen also gave unacceptable rates for termination of pregnancies by curettage with low progesterone values with a significant percentage subsequently carried to term (38%).

This study is the first to use a nonextraction rapid progesterone kit to associate low progesterone values in abnormal pregnancies (ectopic or inevitable abortion) with a control group of similarly presenting pregnancies with abnormal uterine bleeding and abdominal pain who subsequently carried to term. Former studies have used normal pregnancies[1, 3, 4, 6-8] or "patients presenting to the emergency room identified by housestaff as being at risk for ectopic pregnancy."[5, 9] Although this may seem a technicality when determining the usefulness of a testing procedure, the control group must adequately represent the groups requiring differentiation. Radwanska et al.[12] previously documented this finding with an extraction-based, rapid, competitive, protein-binding technique.[13]

In reality, the use of serum progesterone in an attempt to reduce the incidence of ruptured ectopic pregnancies prompts an expedited, complete evaluation in a small proportion of the patients presenting with symptoms that may indicate an ectopic pregnancy. In many cases, as demonstrated in this study, this could be initiated by a high degree of suspicion on the part of the practitioner. In clinical settings where the demand for ultrasonography/laparoscopy may exceed the supply, serum progesterone levels may help determine those most likely to benefit from earlier screening; however, this has not been documented to date. Whether the combination of hCG and progesterone values can prospectively predict an asymptomatic abnormal pregnancy has not yet been adequately addressed. If progesterone levels are obtained in the evaluation of potential ectopic pregnancies, the discriminatory value chosen should be set to detect the majority of ectopic pregnancies (maximal sensitivity, maximal predictive value of a negative test).

Progesterone levels alone in the symptomatic patient should not prompt curettage and add only supportive evidence of an abnormal pregnancy, which prompts further diagnostic procedures (transvaginal ultrasonography or laparoscopy). This retrospective study demonstrates that in two institutions the rate of ruptured ectopic pregnancies as an endpoint would not have been reduced further by the use of progesterone levels than studies using it prospectively; therefore, its utility is questionable.

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The accuracy of auscultatory detection of fetal cardiac decelerations: A computer simulation

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To evaluate current practices of auscultation for the detection of decelerations, we used a computer to generate contractions and late decelerations and perform the counting. The baseline rate ranged from 110 to 180 beats/min. The duration of the deceleration ranged from 1 to 2 minutes, and the amplitude of the deceleration ranged from 10 to 90 beats/min. The onset of the decelerations ranged from 0.4 to 0.7 of the length of the contraction. Counting was begun at 80%, 100%, and 120% of the contraction length. The duration of counting varied between 15 and 60 seconds. A multicount algorithm obtained three 10-second counts separated by 5 seconds. Results were classified by the ability to detect rates below 120, 100, or 80 beats/min (threshold determination) or 20 and 25 beats/min below the baseline rate (subtraction determination). The baseline rate and deceleration amplitude had the greatest effect on accuracy. The higher the baseline rate and the smaller the deceleration amplitude, the less accurate was detection. Multiple counts were more accurate than the single-count strategy, and subtraction detection was more accurate than threshold detection. The effects of counting error are briefly described. This model, which requires clinical confirmation, nevertheless emphasizes the potential inaccuracies of many popular schemes of auscultatory surveillance, even for the detection of prolonged or sustained decelerations. Certain modifications of auscultatory practice may improve the accuracy of this technique. (Am J Obstet Gynecol 1992;166:566-76.)

Key words: Auscultation, decelerations, computer

Auscultation of the fetal heart rate (FHR) as a measure of fetal welfare was introduced into clinical medicine in the second decade of the nineteenth century. The first scientific treatment appeared as an appendix to the second edition of Laennec's treatise on the stethoscope—the "happy idea" of his friend Kergaradec.[1] The first detailed monograph devoted exclusively to fetal auscultation was written by Kennedy in 1845.[2] Although modest, additional enhancements were made over the next century; little research was devoted to the validation of this universally applied but often contradictory technique. Indeed, to this day there has been no systematic study of the impact of auscultation on fetal outcome.

Several reports of the accuracy of clinical auscultation have concluded that obstetric personnel cannot count the FHR accurately or meaningfully, although they

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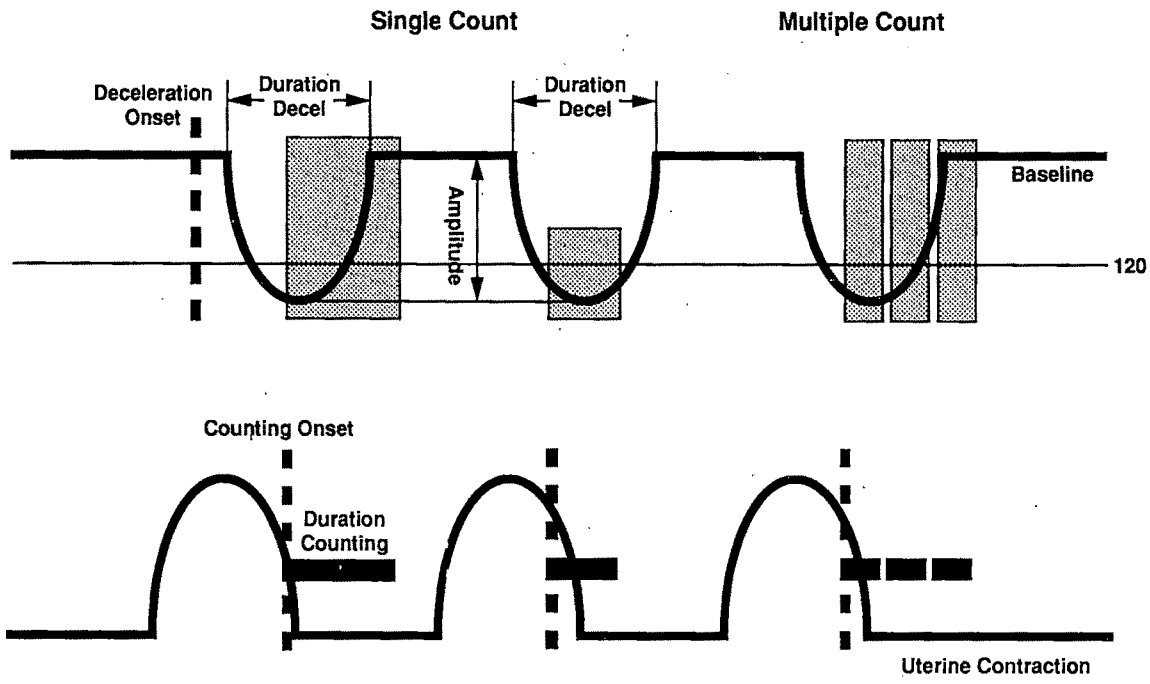


Fig. 1. Components of fetal auscultation. Schematic representation of factors evaluated in this study.

seem to count most accurately when the heart rate is within the normal range.[3-5] The greater the deviation from the average rate, the greater the range of error and the greater the tendency to correct toward the mean. Miller et al.[6] asked experienced labor and delivery physicians and nurses to ascertain the presence of decelerations and other features of the heart rate pattern from fetal heartbeats recorded on tape. Not only were these clinicians unable to reliably identify certain obvious decelerations or features of baseline rate of variability, they could not match auscultatory findings with simultaneous electronic fetal monitor tracings.

In spite of the limitations of auscultation, the results of several recent clinical trials of electronic fetal monitoring purportedly support the notion that auscultation is comparable to electronic monitoring of the FHR.[7, 8] On the basis of these and other studies, the *Guidelines for Perinatal Care* published by the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics[9] seemingly permit auscultation, without electronic fetal monitoring backup, for the evaluation of fetal well-being during labor, irrespective of the risk status of the mother or fetus.

Stimulated by these considerations, we undertook a systematic analysis of clinical auscultation. Rather than compare, as others have, the practical accuracy of counting or the ability to detect decelerations under test or clinical conditions, we chose a different model with which to begin to examine the problem. Using computer simulation, we evaluated the impact of current clinical practices of auscultation on the detection of decelerations. We systematically explored the effect of varying the baseline rate, along with the amplitude, onset, and duration of decelerations on the detection of decelerations. We also explored the impact of varying the onset and duration of counting, and we briefly illustrate the impact of counting error. Analysis of this model suggests that features of the deceleration and, to some extent, the duration of auscultation may profoundly influence the detection of decelerations. Certain modifications of auscultatory practice, however, may improve its accuracy.

Material and methods

For this evaluation all simulations and counting were done by desktop computer (IBM-PC Model AT). The parameters were established by the investigators to encompass the range of heart rate features customarily encountered in clinical practice and to attempt to duplicate popular schemes of clinical surveillance. Fig. 1 provides a schematic illustration of the test protocol. Table I enumerates the values used for the independent variables. The computer generated both contractions and decelerations according to the following scheme: The length of the uterine contraction was normalized to a duration of one minute. The contraction amplitude was not varied. The baseline rate ranged from 110 to 180 beats/min. The onset and return to baseline of all decelerations were symmetric and plotted

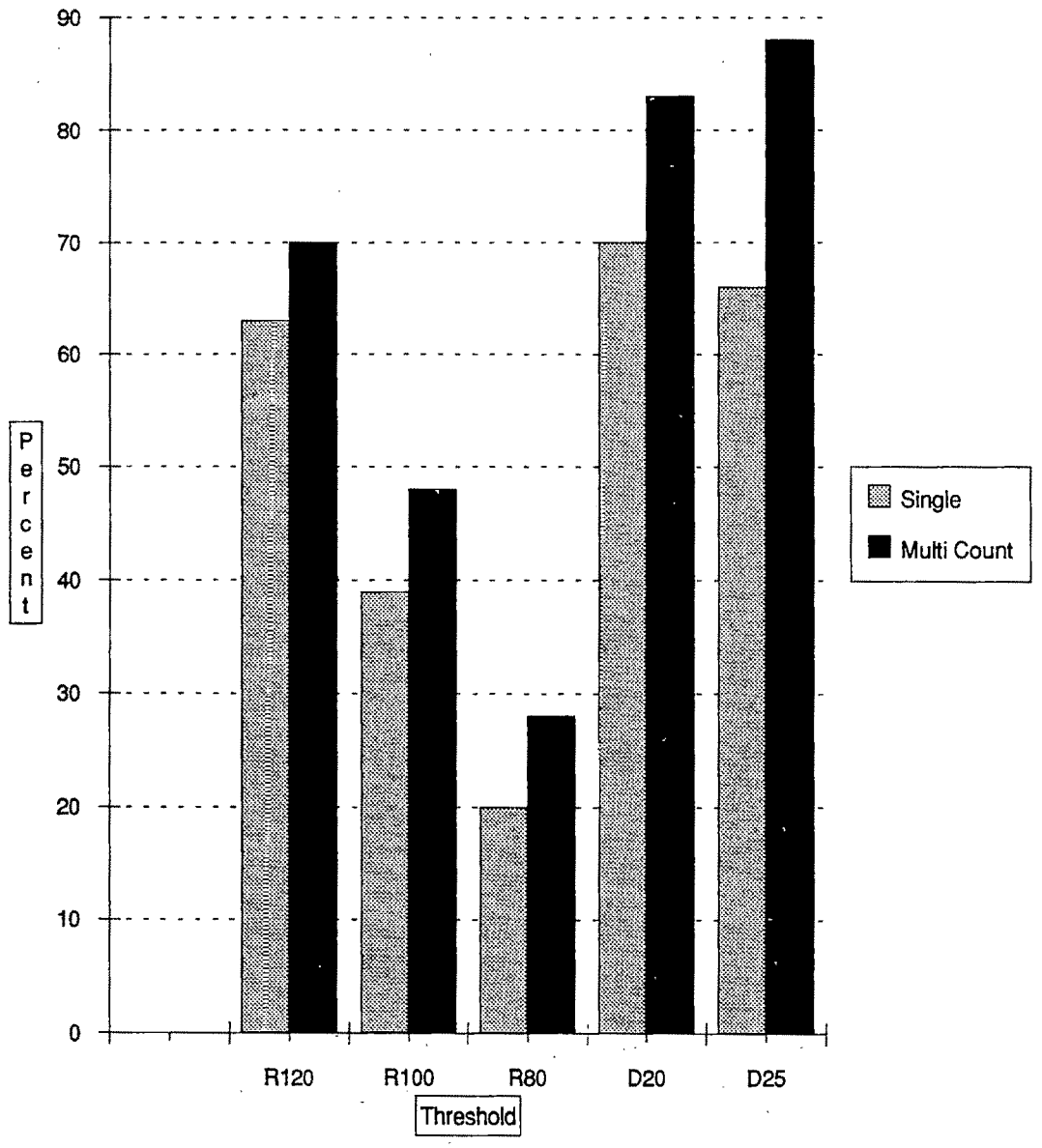


Fig. 2. Detection of decelerations according to threshold for single and multicount cases. In each instance multicount technique detects a greater proportion of decelerations than does single-count technique. *R120,* Threshold 120 beats/min; *R100,* threshold 100 beats/min; *R80,* threshold 80 beats/min; *D20,* threshold 20 beats/min below baseline; *D25,* threshold 25 beats/min below baseline.

as a sine function of the heart rate. The duration of the deceleration ranged from 1 to 2 minutes, and the amplitude of the deceleration ranged from 10 to 90 beats/min. The onset of the decelerations ranged from 32 to 42 seconds into the uterine contraction (0.4 to 0.7 of the length of the contraction). Depending on their duration and amplitude, the decelerations would be classified as either late decelerations, variable decelerations with slow return, or prolonged decelerations.

Because the end of the uterine contraction will vary depending on whether contractions are determined by patient perception, palpation of the abdomen, or electronic tocodynamometry, we initiated counting at 80%, 100%, 120%, and 150% of the contraction, corresponding to 48, 60, 72, and 90 seconds after the onset of the prototypic (1-minute) contraction. The duration of counting varied between 15 and 60 seconds; the computer made the appropriate multiplication to obtain the rate in beats per minute. We also implemented a multicount algorithm as follows: Instead of a single count as described, we obtained three 10-second counts separated by 5 seconds between counts. The lowest of the three counts was considered the detected rate.

Table I. Values of independent variables used in study

Baseline rate (beats/min)	110, 120, 130, 140, 150, 160, 170, 180
Decelerations	
Duration (min)	1.0, 1.5, 2.0
Amplitude (beats)	10, 20, 30, 40, 50, 60, 70, 80, 90
Onset (min after onset of uterine contraction)	0.40, 0.55, 0.70
Counting	
Onset (min after onset of uterine contraction)	0.8, 1.0, 1.2, 1.5
Duration	
Single count (sec)	15, 30, 60
Multicount (No. of 10 sec counts)	3
Contraction	
Duration (min)	1

Table II. Detection of amplitude of decelerations according to parameter

Parameter	*Single count*	*Multicount*	*Parameter*	
			High	*Low*
Baseline	74	93	110	180
Deceleration	74	93	90	10
Duration of deceleration*	83, 59	97, 90	2.0	1.0
Start of deceleration*	76, 70	96, 91	0.4	0.77
Duration of count*	85, 59	—	15	60
Start of count	74	93	0.8	1.5
Average	74	93	–	—

*High and low

There is no uniform agreement as to which heart rate shall be accepted as indicative of a fetal deceleration. Although 120 beats/min is generally accepted as the lower limit of normal, some recommend using 100 or 110 beats/min, others 80 beats/min. Accordingly, we have classified the results according to the ability to detect rates below 120, 100, or 80 beats/min (threshold determination). In addition, we also explored the impact of making the critical level of detection 20 and 25 beats/min below the baseline rate. This involved subtracting the detected rate from the baseline rate (subtraction determination).

Because this model is based exclusively on parameters established by the investigator (a deterministic model), the results are presented simply according to the amount of the deceleration detected and, more importantly, the frequency of detection of decelerations for each parameter studied. The dependent variable, deceleration detected, was defined as 1 for those cases in which the calculated rate during the period of counting satisfied the selection criteria and 0 otherwise. The duration of counting was excluded in the multicount examples. We emphasize that the program distributed all the independent variables evenly across the chosen range; that is, there were equal numbers of decelerations at 10, 30, 50, and 70 beats/min and equal numbers lasting 1, 1.5, and 2 minutes. This does not reflect the distribution of decelerations in clinical practice (see subsequent text).

We briefly explored the impact of erroneous counting on the detection of decelerations. We limited the potential error to the addition and/or subtraction of one or two beats to the count, corresponding to an error of one beat at the beginning and/or end of the count. The potential errors of counting may be represented as follows:

$$\text{Calculated rate} = \frac{\text{True count } (\pm 2 \text{ beats}) \cdot 60}{\text{Duration of counting (seconds)}}$$

Results

For the evaluation of this model, we generated 10,368 records, including 7776 single-count and 2592 multiple-count records. For the single-count strategy, counting detected, on average, about 74% of the amplitude of the deceleration with a range of 59% to 85% according to the independent variable. For the multicount strategy, counting detected on average 93% of the amplitude to deceleration, with a range of 91% to 97% (Table II). Identifying a drop in heart rate as a deceleration, however, was influenced dramatically by the threshold criteria (120, 100, or 80 beats/min or a drop of 20 or 25 beats/min), the technique of counting (single count or multicount), the features of the deceleration, and the features under the control of the listener (Table

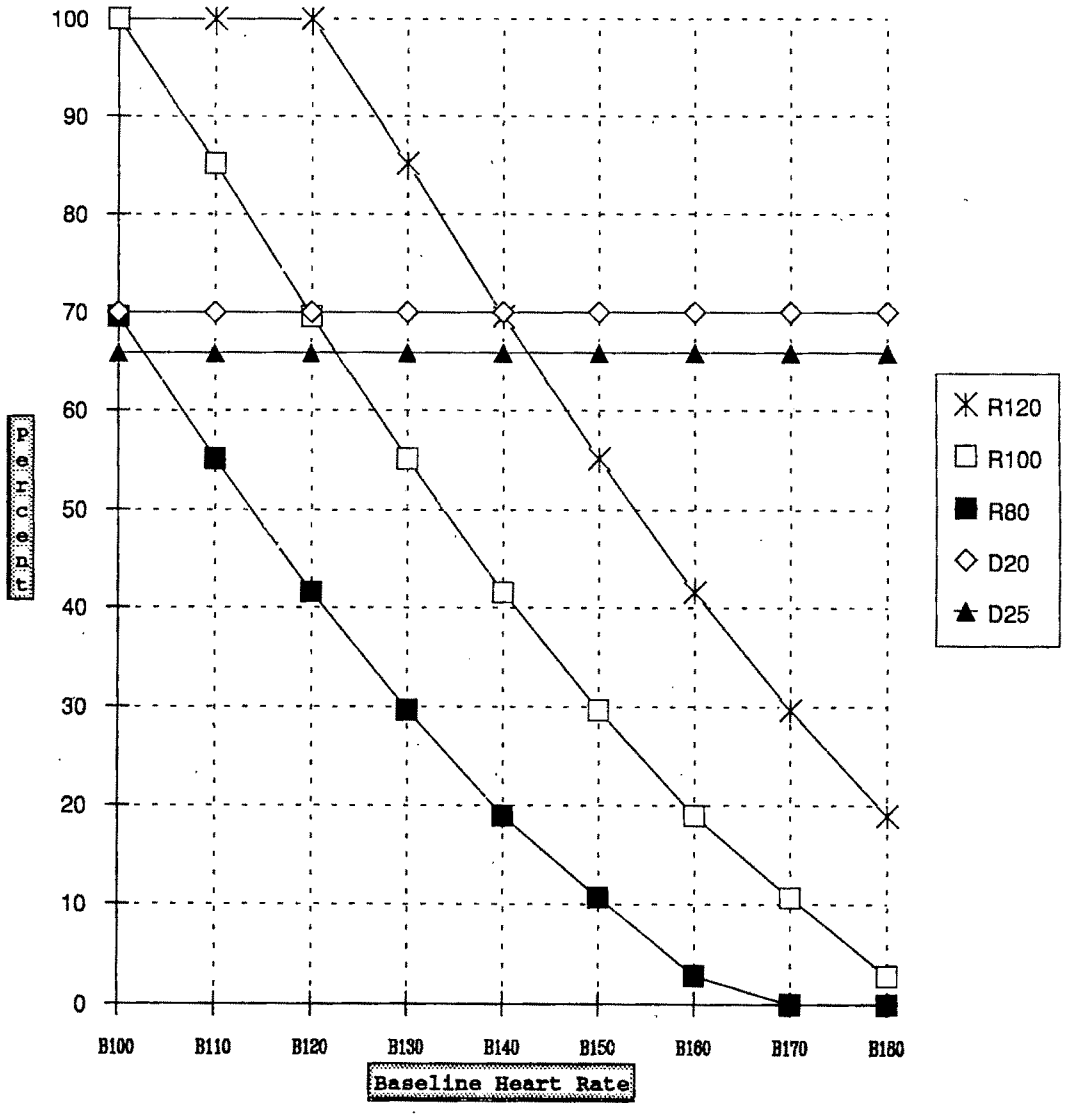


Fig. 3. Detection of decelerations according to baseline heart rate and counting threshold. When threshold involved fixed heart rate (i.e., 100 beats/min), then the higher the baseline rate, the lesser was the accuracy of detection. For subtraction techniques *(D20, D25)*, accuracy was not affected by baseline rate.

Table III. Detection of decelerations according to parameter

Parameter	*Single count*	*Multicount*	*Parameter*	
			High	*Low*
Baseline	85, 3	89, 9	110	180
Deceleration*	76, 0	97, 0	90	10
Duration of deceleration*	45, 30	50, 48	2.0	1.0
Start of deceleration*	40, 37	50, 48	0.4	0.77
Duration of count	45, 30	—	15	60
Start of count	39	49	0.8	1.5
Average	39	49	–	—

*High and low.

III, Fig. 2). The baseline heart rate and the amplitude of the deceleration had the greatest effect (Figs. 3 and 4). The higher the baseline rate and the smaller the amplitude of the deceleration, the fewer decelerations were detected (Table IV). The longer the duration of the deceleration and the later its onset in the contraction cycle, the more readily was the deceleration detected (Fig. 5). The start of the deceleration played only a small

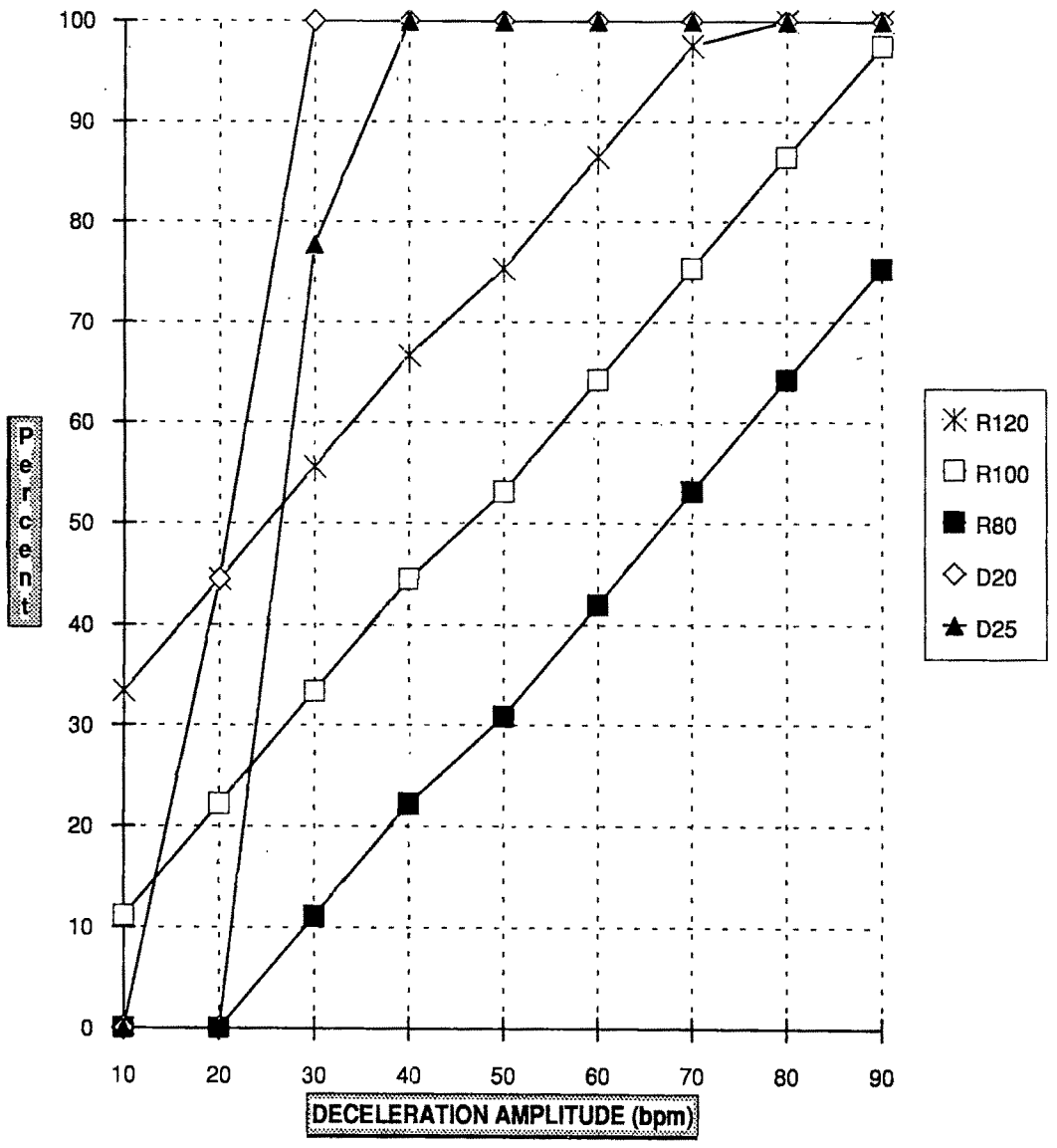


Fig. 4. Detection of decelerations according to deceleration amplitude and counting threshold. Irrespective of counting threshold, the greater the deceleration amplitude, the greater was the accuracy of detection.

role in the detection of decelerations and the determination of their amplitude. The onset of deceleration played no role in the detection of decelerations.

Factors under control of the examiner had limited impact on the detection of decelerations. Early onset of counting was somewhat more accurate than later onset, but this depended on features of the deceleration. Beginning to count after the end of the contraction (1.5 minutes) was only effective when decelerations were prolonged. Shorter counts were more accurate than longer ones (Fig. 6), but this depended on the duration of the deceleration. If the deceleration were relatively short and the duration of counting long, a considerable amount of baseline period would be counted and most decelerations would be missed. On the other hand, with prolonged decelerations the duration of counting did not have an impact on the frequency of detection.

As expected, for every parameter, the higher the threshold, the more decelerations were detected. Fig. 2 compares the detection rate for each threshold level. There is about a 20% reduction in detection as the threshold is lowered by 20 beats/min. Similarly, for every parameter multicount was better than single count. It provided only marginal improvement, however, over the early (0.8 or 1.0 minute), 15-second count. Because 92% of the minimal counts were obtained in either the first or the third epoch of counting, dropping the second 10-second counting period made little practical difference in the detection frequency.

When the basis for the detection of a deceleration involved a drop of 20 or 25 beats/min from the base-

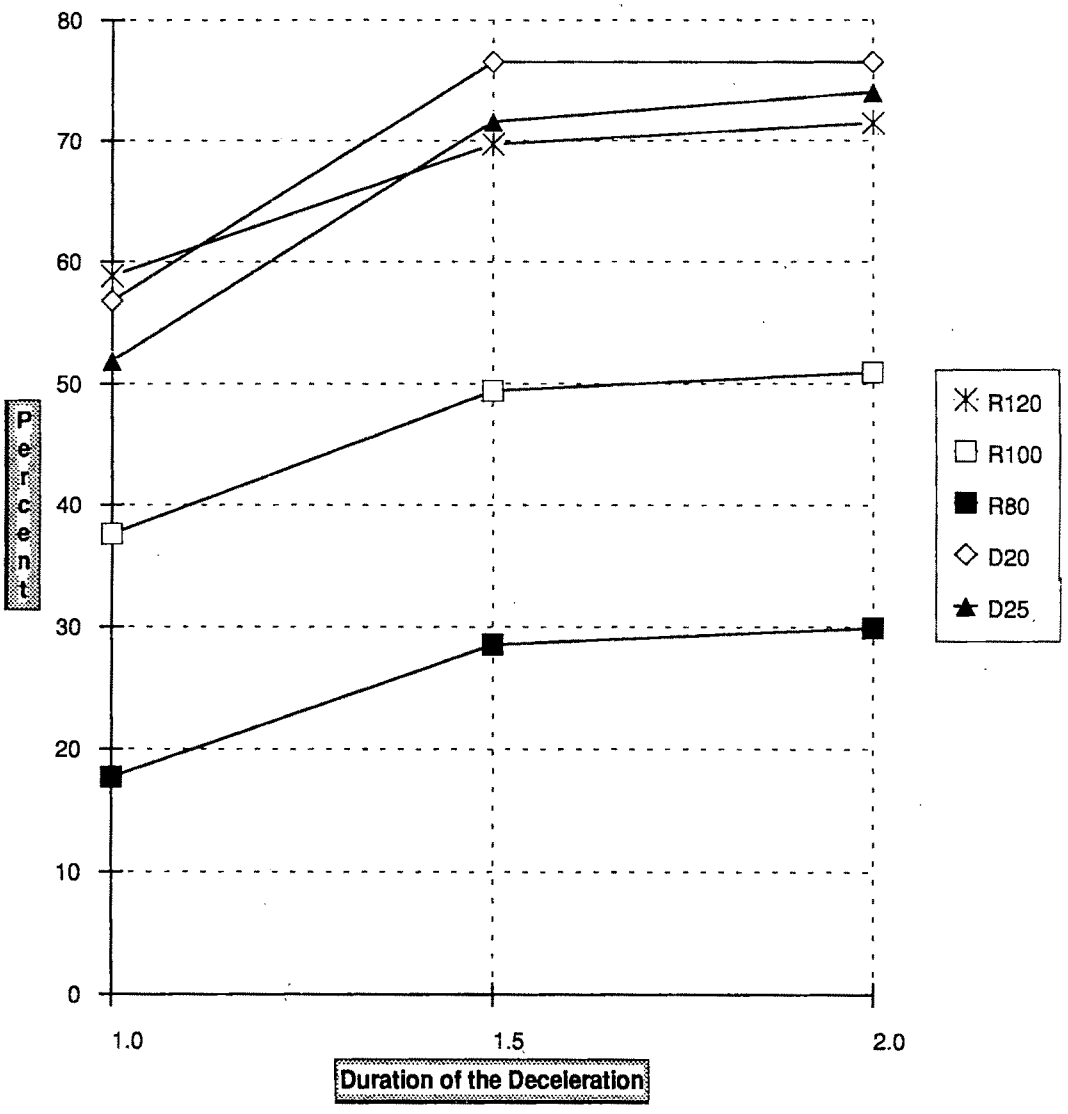


Fig. 5. Detection of decelerations according to duration of decelerations and counting threshold. The longer the duration of the deceleration, the greater the accuracy of detection.

Table IV. Detection of decelerations: Influence of baseline rate and deceleration amplitude

Baseline (beats/min)	*Threshold of 100 beats/min (%)*								
	10	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*
110	0	82	93	96	96	100	100	100	100
120	0	0	70	85	89	93	96	96	96
130	0	0	0	63	82	85	85	89	93
140	0	0	0	0	52	70	82	85	85
150	0	0	0	0	0	44	63	78	82
160	0	0	0	0	0	0	37	63	70
170	0	0	0	0	0	0	0	37	59
180	0	0	0	0	0	0	0	0	26

line, detection accuracy improved considerably but only at higher baseline rates (Figs. 2 and 3). Although this strategy, however, can detect >75% of decelerations of 30 beats/min in amplitude, it detects none of lesser amplitude. Ultimately, in this model the most accurate technique combined the multicount strategy with the 20 to 25 beats/min drop in heart rate from the baseline.

Table V lists the potential deviation for one or two

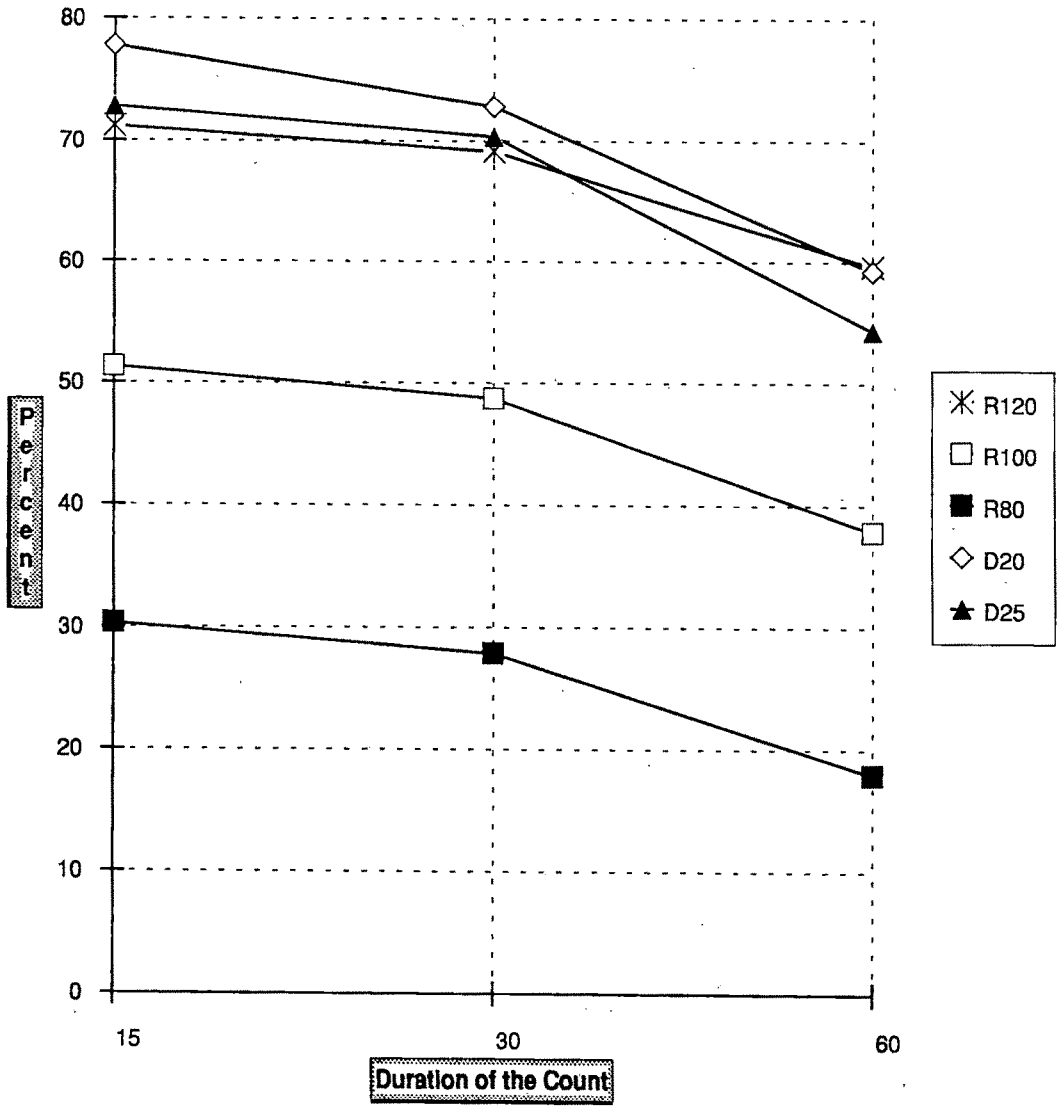


Fig. 6. Detection of decelerations according to counting duration. The longer the count, the lesser the accuracy.

beats of error per counting period. The shorter the count, the larger the multiplier and the greater the potential error. The error, however, is unrelated to the actual FHR. The impact on detection of decelerations according to the duration of counting is illustrated in Fig. 7.

Comment

We again emphasize that the model presented here is based on simulated and not clinical data. It is a limitation of this study that we have not attempted to match the distribution of baseline rates and the features of these decelerations to a clinically determined distribution. We are unaware of such an analysis, nor are we aware of any attempt to determine the consistency and reliability of the clinical detection of the end of the contraction. Those articles dealing with the accuracy of auscultation show a considerable bias in the way the heart beats are counted, especially at high and low rates.[3-6] We have not dealt here with sinusoidal patterns, decreased variability, accelerations, other types of decelerations, or those patterns associated with neurologic handicap. These electronic fetal monitoring patterns rarely involve severe decelerations and seemingly cannot reliably be detected by auscultation.[6, 10-13] These considerations underscore our belief that the data presented here are important for their trends, not for the actual percentages.

Nevertheless, these results bring into focus the potential problems of many popular schemes of auscultatory surveillance, even for the detection of deep or sustained decelerations. The detection of decelerations

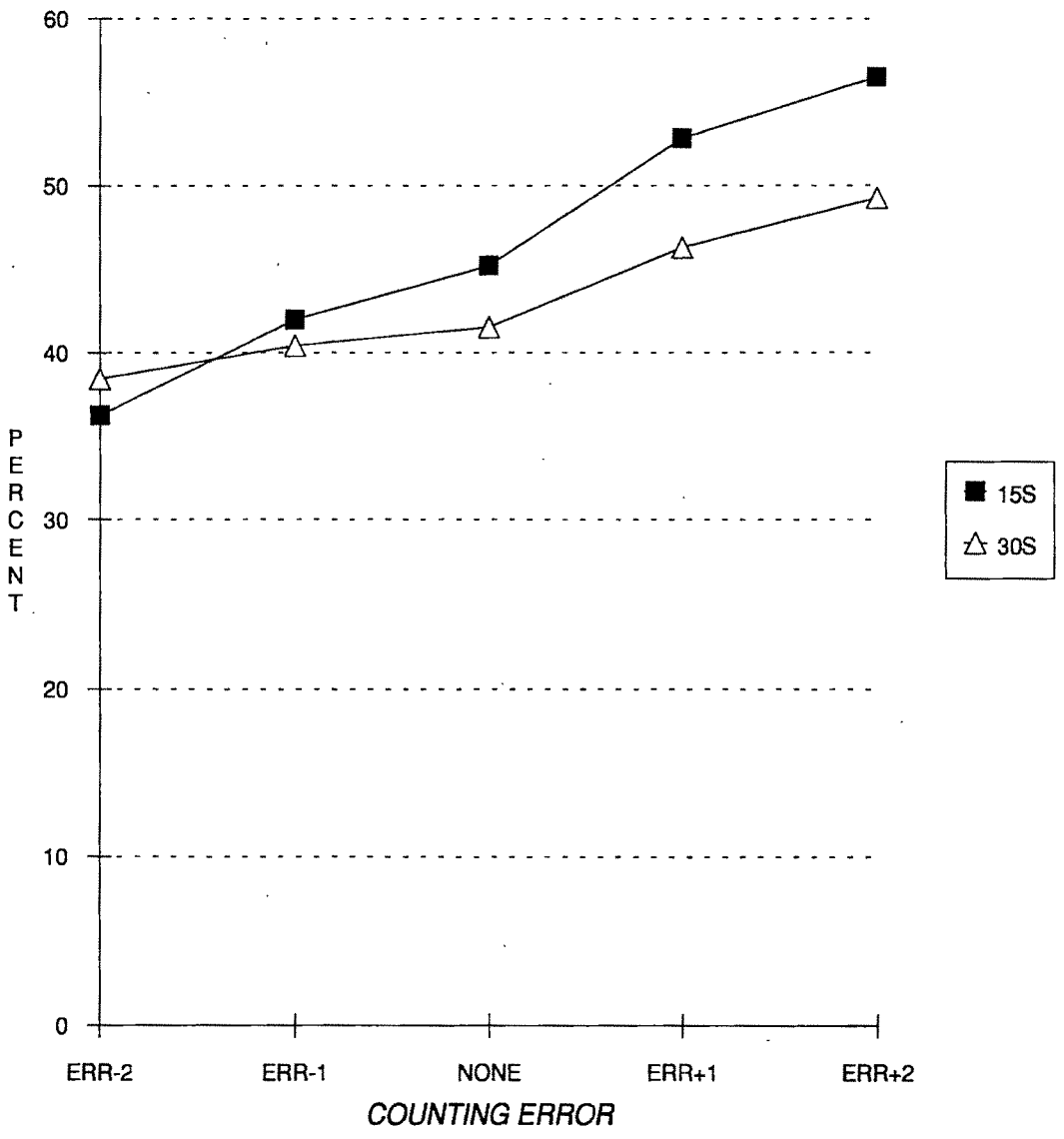


Fig. 7. Effect of counting error on accuracy of detection of decelerations. The shorter the count, the greater was the potential error (see text).

varies considerably, depending on the technique of counting (single count, multicount), the threshold criteria (120, 100, or 80 beats/min or a drop of 20 or 25 beats/min), along with the baseline rate and amplitude of decelerations, and, to a lesser extent, the onset and duration of the deceleration. While factors under the control of the health care provider had only a small effect on accuracy, nevertheless, detection accuracy deteriorates with increasing delay in the onset of counting and with increasing duration of counting.

The results of this model provide no support for counting fetal heartbeats during 1 minute. Unless decelerations are prolonged, this duration of counting diminishes the yield. While multiple, short-lived counts were most accurate, single, short counts (e.g., 15 seconds) were only minimally less accurate. In this respect we have briefly considered the potential impact of counting error on this simulation. Although the shorter counting periods were most accurate in this simulation, this advantage is potentially offset by the greater potential error in counts of short duration. The 10-second count used for the multiple count would be expected to carry the greatest potential error, but this may be offset by the repetitive counts.

Compared with the use of a threshold level of heart rate (e.g., 120 beats/min) to determine fetal decelerations, a critical drop in rate from the baseline seems to provide a more reliable detection scheme, especially if

the baseline rate exceeds 140 beats/min. While the accuracy of detection is improved by this strategy, even this approach will miss about 25% of the decelerations of 30 beats/min amplitude. It may not be possible to optimize auscultation for the detection of smaller decelerations, because the likelihood of error or normal variation will doubtless exceed by manyfold the detection of fetal decelerations. This is not to say that small decelerations are insignificant, only that their reliable detection, in this model at least, seems insurmountable. Whether the critical level should be established at 20 or 25 beats/min or some other value below the established baseline must be determined clinically.

Both these data and the potential errors of counting need to be confirmed in clinical studies. If the model proves accurate, it suggests the need for a fundamental shift in the acquisition and approach to auscultation data. The importance of the baseline rate in assessing decelerations cannot be overemphasized. If the baseline rate is <120 beats/min, as prevails in a number of postdate pregnancies, the normal variation in counting will needlessly alarm the physician or nurse every time the heart rate is counted, irrespective of the presence of decelerations. With high baseline heart rates, the determination of decelerations with the threshold technique becomes insurmountable. At a baseline rate of 160 beats/min, considered the upper range of normal, only half of the decelerations of 50 beats/min were detected at a threshold of 120 beats/min; none of lesser amplitude were detected. At a baseline rate of 140 beats/min, considered the average heart rate, only half of the decelerations of 50 beats/min were detected at a threshold of 100 beats/min; none of lesser amplitude were detected.

In a controlled trial comparing auscultation with no auscultation in 1951, Walker[14] failed to show any relationship between auscultated FHR and neonatal outcome. In this paper, the author also summarized the published contradictions of auscultatory criteria of fetal distress. A study by Benson et al.[15] involving over 25,000 patients concluded that intermittent auscultation of the FHR was of no demonstrable value in the prediction of early fetal distress. Williams' textbook[16] discusses the limitations of this auscultatory approach (counting begun 30 seconds after the contraction), a feature that had little impact on accuracy in this study, but fails to cite evidence affirming the hypothesis that auscultation improves fetal outcome by the early detection of fetal distress. Apparently paradoxically, several studies have concluded that auscultatory signs of fetal distress are not a risk factor for poor outcome.[17, 18] Data from The Collaborative Project found a greater incidence of low Apgar scores among patients in dysfunctional labor than among those with fetal distress.[18] Neither the American College of Obstetricians and Gynecologists and American Academy of Pediatrics guidelines nor Williams' textbook defines features of auscultation such as the duration of counting, the critical levels for intervention, or the establishment of baseline rate.

Table V. Deviation in detected rate according to duration of count and counting error

Duration of count (sec)	*Error (± beats/min)*	
	1 Beat	*2 Beats*
10	6	12
15	4	8
20	3	6
30	2	4
40	1.5	3
50	1.2	2.4
60	1	2

We will not comment further here on the benefits of auscultation and electronic fetal monitoring. We only wish to remind the reader that auscultation has not been subjected to the same critical review as has electronic fetal monitoring. Perhaps a more scrupulous approach to the acquisition of auscultatory data would improve this situation.

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Successive pregnancies complicated by idiopathic sideroblastic anemia

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Sideroblastic anemias are a diverse group of hypoproliferative anemias characterized by defective iron use within erythropoietic cells and a defect in heme biosynthesis. This report describes the first case of successive pregnancies in a patient with idiopathic sideroblastic anemia. Periodic transfusions with washed erythrocytes and oral pyridoxine resulted in normal maternal and perinatal outcome. (AM J OBSTET GYNECOL 1992;166:576-7.)

Key words: Sideroblastic anemia, pregnancy, pyridoxine

Sideroblastic anemia is a descriptive term for a diverse group of disorders that have in common a defect in heme biosynthesis and defective iron use within erythropoietic cells. A sideroblast is a marrow erythroid precursor (specifically, a normoblast) that has Prussian blue–positive granules within the cytoplasm. The granules represent deposits of iron phosphate within mitochondria, which have not been used for heme synthesis. The abnormal accumulation may form a perinuclear ring of iron. This ringed sideroblast is the hallmark of sideroblastic anemia.[1]

Sideroblastic anemia may be hereditary or acquired. Both sex-linked and autosomal recessive patterns of inheritance have been described. The acquired variety of sideroblastic anemia has been associated with certain diseases (especially malignant and inflammatory disorders) and some drugs or toxins (especially ethyl alcohol, lead, isonicotinic acid).[2] There is also an idiopathic variety of sideroblastic anemia.

Sideroblastic anemia is an exceedingly unusual complication of pregnancy. Because of our inability to identify a report of successive pregnancies associated with idiopathic sideroblastic anemia, the management of the following patient is presented.

Case report

A 22-year-old black woman, gravida 2, para 1, was seen at 10 weeks' gestation. Two years earlier, prenatal evaluation had revealed severe anemia. At that time, the hemoglobin was 5.8 gm/dl and hematocrit 19.1%. There were 2,010,000 erythrocytes and 310,000 platelets per cubic millimeter. Reticulocytes were 0.7%. Leukocytes and platelets were normal. Smear of peripheral blood revealed a mixture of normocytic-normochromic cells and hypochromic-microcytic erythrocytes. Anisocytosis, poikilocytosis, target cells, schistocytes, microcytes, and rare basophilic stippling were demonstrated. There were no Pappenheimer bodies.

On bone marrow biopsy, the marrow-to-fat ratio was 70:30. Red blood cells showed erythroid hyperplasia

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with increased numbers of prorubricytes and rubricytes. Progressive hemoglobinization was seen. Special stains for iron showed a marked increase in sideroblasts. The majority of sideroblasts demonstrated perinuclear ring distribution of coarse granules. There was no other abnormality.

The diagnosis was sideroblastic anemia. There was no hereditary predisposition, drug-toxin exposure, and no associated disease. The anemia did not respond to pyridoxine therapy (50 mg daily). Intermittently, the patient required transfusions of washed erythrocytes to maintain the hemoglobin in the 9 to 10 gm/dl range. She experienced spontaneous onset of labor at term but required cesarean section for cephalopelvic disproportion. Transfusion was necessary at the time of surgery. The postoperative course was uneventful. Three weeks after surgery, the hemoglobin was 7.9 gm/dl and hematocrit 19%. She was lost to follow-up until 3 months post partum. At that time, the hematocrit was 11.2%, and again she required transfusions.

The patient used no form of contraception and failed to keep appointments until she suspected pregnancy. When seen at 10 weeks' gestation in the subsequent pregnancy, hemoglobin was 4.7 gm/dl and hematocrit 13.4%. She was transfused with two units of washed erythrocytes and begun on 200 mg pyridoxine daily and 2 mg folate daily. The pregnancy proceeded in an uneventful fashion except for the requirement of intermittent transfusions. Antepartum evaluation of fetal well-being with serial ultrasonography and nonstress testing were normal. There was never any evidence of antibody production as a result of the repeated blood transfusions. After the spontaneous onset of labor at 37 weeks' gestation, a repeat cesarean section and tubal sterilization were performed. The female infant weighed 2296 gm with Apgar scores of 9 and 9. There were no complications. Postoperative hematocrit was 29.5%.

Comment

Anemia is frequently encountered in pregnancy. On occasion, a chronic anemia will require bone marrow analysis. The finding of ringed sideroblasts is diagnostic of sideroblastic anemia. This report demonstrates one patient with idiopathic sideroblastic anemia who was managed successfully throughout two pregnancies by periodic transfusions of washed erythrocytes and oral pyridoxine therapy.

Management is directed toward maintenance of the hematocrit by periodic transfusions of washed erythrocytes. Obvious potential complications include isoimmunization and viral infections. Although only a minority of patients with idiopathic sideroblastic anemia respond to pyridoxine, patients should be treated with 100 to 200 mg of pyridoxine per day (about 100 times the physiologic daily requirement). Further, oral folate supplementation (1 to 2 mg per day) is recommended because concomitant folate deficiency is frequent with idiopathic sideroblastic anemia. Because sideroblastic anemia is allied with myelodysplastic syndromes, patients should also be monitored for the development of acute leukemia.

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Dysmenorrhea and use of oral contraceptives in adolescent women attending a family planning clinic

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OBJECTIVES: This study examined the prevalance of dysmenorrhea in female adolescents and the effect of experiencing a reduction in dysmenorrhea on oral contraceptive use.

STUDY DESIGN: This was a prospective panel study in which 308 adolescent women at an inner-city family planning clinic were interviewed about their experiences with dysmenorrhea and their oral contraceptive use at three points in time over a 6-month period. A χ^2 test and multiple logistic regression analysis were done.

RESULTS: The overall prevalence of dysmenorrhea in this population was 79.6%; 18.2% reported severe dysmenorrhea. Those who had severe dysmenorrhea and also experienced the reduction of dysmenorrhea as a result of oral contraceptives were eight times more likely to be consistent oral contraceptive users ($p \leq 0.02$).

CONCLUSIONS: It is important to screen female adolescents for dysmenorrhea, provide them with information about the beneficial side effects of oral contraceptives, and follow up these young women to make sure they are experiencing the alleviation of their symptoms. (Am J Obstet Gynecol 1992;166: 578-83.)

Key words: Dysmenorrhea, oral contraceptives, adolescents

Factors influencing effective oral contraceptive use among young women, and in particular adolescents, have been extensively researched. Age, degree of sexual activity, adverse effects, and attitudes of clinic personnel have all been examined, and varying degrees of impact have been ascribed. Missing, for unknown reasons, is the impact of dysmenorrhea. Primary dysmenorrhea is an important clinical and social problem affecting more than 50% of menstruating women.[1] Factors associated with and influencing this problem have been carefully reported in a study of over 600 young women at age 19 and again in the same women at age 24.[2-4] Time, parity, and the use of oral contraceptives were found to be associated with reduction in pain and improved physical and mental attitude. The beneficial impact of oral contraceptives is well established.[4-6] It was the reverse question (does reduction of dysmenorrhea favorably influence oral contraceptive use?) which prompted the inclusion, in a recent study of adolescents' contraceptive use, of an investigation of dysmenorrhea.

This paper reports the results of an analysis of the impact of dysmenorrhea and the experienced reduction of dysmenorrhea on oral contraceptive use over a 6-month period in a panel of adolescent clients of an inner-city family planning clinic.

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Material and methods

Subjects. The data for this analysis are from a three-wave panel study of female adolescents who obtained prescriptions for oral contraceptives at a Planned Parenthood clinic in Baltimore during 1988. These women underwent follow-up for 6 months regardless of any return visits to the clinic. At baseline, 430 women were enrolled in the study and interviewed immediately after the clinic visit. Eligibility was limited to those aged ≤18 years who were unmarried, not pregnant, and attending the clinic for purposes of initiating contraception (in some cases after an abortion). The enrolled subjects were 72% of all those eligible during 1988. The major reason for refusing to participate at baseline was reluctance to spend additional time in the clinic. There were no differences in participation rates by age or reason for clinic visit.

Two follow-up interviews were conducted by telephone 3 and 6 months after each subject's baseline interview. Eighty-nine percent (n = 382) of baseline respondents completed both follow-up interviews, and there were no differences in completion rates by age, race, or reason for the initial clinic visit. The major reason for attrition was failure to locate the respondent.

This analysis focuses on a subset of the panel (n = 308) who reported at least one instance of sexual

Table I. Verbal scoring system for assessment of severity of dysmenorrhea*

Severity	*Sometimes or always experience cramps*	*Cramps are very painful*	*Sometimes or always cut back on activities*
No dysmenorrhea	No	No	No
Mild dysmenorrhea	Yes	No	No
Moderate dysmenorrhea	Yes	Yes	No
Severe dysmenorrhea	Yes	Yes	Yes

*The questions used in constructing this scale are: (1) How often do you usually get cramps while you are having your menstrual period—always, sometimes, or never? (2) Would you describe these cramps as very painful or a little painful? (3) On the days when you are having your period, how often do you cut back on your regular activities like staying home from school or staying away from work? Would you say always, sometimes, or never?

intercourse during the follow-up period. These subjects were primarily black (77%), resided in Baltimore City (89%), and were 16 years old on average. Twenty-two percent of them had mothers who were not high school graduates, 58% had mothers who were themselves teenage mothers, and 39% lived with both their mother and a father or stepfather.

For those subjects who had initiated sexual activity by baseline ($n = 293$), the mean age at first intercourse was 14.5 years, and they had an average of 2.9 sex partners. Pregnancy was not uncommon; 58% had been pregnant and 12% had given birth. In general, these subjects are typical of an inner-city population in which sexual activity is initiated at a relatively young age and teenage pregnancy is prevalent.

The interviews were conducted by female interviewers employed by Survey Research Associates, Inc., and not affiliated with the clinic. The baseline interview included questions about sexual and contraceptive history, including ones about the severity and frequency of dysmenorrhea. The two follow-up interviews contained detailed questions about instances of vaginal sexual intercourse and contraceptive use. Additionally, each respondents' clinic record was abstracted at the end of her 6-month follow-up period to obtain information on return visits to the clinic and pregnancies.

Statistical methods. Significance tests for relationship between the severity of dysmenorrhea and subject characteristics and sexual, contraceptive, and health behaviors were calculated with the χ^2 test. The change in odds of being a consistent oral contraceptive user for each variable considered was calculated with multiple logistic regression. The improvement χ^2 test was used to determine the significance of individual predictors in the regression model.

Dependent variable. The dependent variable in this analysis is consistency of oral contraceptive use during the 6-month follow-up period, as reported by the respondent in the follow-up interviews. An extensive discussion of this measure has been reported elsewhere.[7] Briefly, on the basis of information on the date of the index clinic visit, clinic instructions regarding when to start the first package of pills and the respondent's reported pill use (including missed pills), the measure computes the number of days on which each respondent used oral contraceptives divided by the total number of days it was possible for her to have taken oral contraceptives. On average, subjects used oral contraceptives on 65% of possible pill days during the follow-up period; nine subjects never initiated oral contraceptive use, and 19 subjects reported perfect oral contraceptive use.

Because the measure of oral contraceptive use was skewed toward perfect use, it was dichotomized for analysis on the basis of clinical significance of missed pills, according to clinic protocol.[8] Subjects missing three or fewer pills per pack were classified as consistent pill users (38% of all subjects) and those missing more than three pills per pack were classified as inconsistent users (62% of all subjects).

Validation for the dichotomized measure of consistent oral contraceptive use was confirmed by significant associations with several criterion variables. Compared with inconsistent oral contraceptive users, consistent users had significantly more return visits to the clinic for additional prescriptions (58% vs 40%, $p \leq 0.05$), significantly fewer acts of unprotected intercourse (2% vs 39%, $p \leq 0.05$), significantly fewer pregnancy scares (20% vs 33%, $p \leq 0.05$), and fewer pregnancies (6% vs 12%, $p \leq 0.08$).

Dysmenorrhea severity scale. The scale used in this analysis to measure the severity of dysmenorrhea is based on the one created by Andresch and Milsom[2] in a study of 19-year-old women. It ranks the severity of dysmenorrhea using information on how often the women experiences menstrual cramps, how painful she perceives them to be, and how often she cuts back on activities because of them (Table I). This information was gathered at the baseline interview and refers to the time period before oral contraceptive use was initiated and, for the postabortion patients, before the pregnancy resulting in the abortion at the index visit. The main difference between this scale and the Andresch and Milsom scale is that this one does not incorporate the use of analgesics as part of the measure.

Experiencing any reduction in severe dysmenorrhea due to oral contraceptive use was hypothesized to be related to consistent use of oral contraceptives. It was measured by asking respondents about positive side effects of oral contraceptives during the first 3 months

Table II. Prevalence and severity of dysmenorrhea in female adolescents attending a family planning clinic (n = 308)

Severity	*Score*	*n*	*%*	*Cumulative percent, any dysmenorrhea*
No dysmenorrhea	0	63	20.5	0
Mild dysmenorrhea	1	141	45.8	45.8
Moderate dysmenorrhea	2	48	15.6	61.4
Severe dysmenorrhea	3	56	18.2	79.6

Table III. Dysmenorrhea by subject characteristics

Characteristic	*Severity*				*All subjects*	*p Value*
	0	*1*	*2*	*3*		
Demographic factor						
Mean age (yr)	16.3	16.1	16.1	16.1	16.2	1.00
Black (%)	76.2	75.2	81.3	76.8	76.6	0.86
Mother's education (did not graduate from high school) (%)	22.2	18.4	22.9	26.8	21.4	0.62
Parous before baseline (%)	22.2	12.1	4.2	8.9	12.3	0.03
Early onset of menstruation (before age 12) (%)	24.6	31.2	47.9	33.9	33.0	0.07
Sexual/contraceptive behavior						
Consistent pill users (%)	38.1	37.6	35.4	42.9	38.3	0.88
Experiencing positive side effects (%)	55.6	66.7	76.6	69.6	66.0	0.12
Intercourse at least once a week on average* (%)	27.0	33.3	35.4	41.1	33.8	0.44
Somewhat or very happy about sex† (%)	69.8	74.5	62.5	62.5	69.5	0.26
Intercourse is sometimes or always painful‡ (%)	14.3	27.0	29.2	37.5	26.6	0.04
Health behavior (%)						
Return clinic visit§	33.3	46.8	60.4	48.2	46.4	0.04
Called clinic during follow-up	25.4	30.5	33.3	25.0	28.9	0.70
Drink alcohol once a month or more	15.9	24.8	22.9	19.6	21.8	0.52
Smoke once a month or more	12.7	22.0	18.8	32.1	21.1	0.08

*A count of the instances of vaginal intercourse reported was dichotomized at the mean (mean = 24 acts or an average of once a week).

†Subjects were asked at baseline how they felt about having intercourse. Responses were on a five-point scale, ranging from very unhappy to very happy.

‡Subjects were asked at baseline if they ever experienced pain during intercourse, and if so how often it occurred. Responses were "almost never hurts," "sometimes hurts," "almost always hurts."

§This is a return visit, to obtain oral contraceptives, to the same clinic where oral contraceptives were initially obtained.

of use and combining this information with their baseline reports of dysmenorrhea. Positive side effects measured in the first follow-up interview were reduced menstrual cramping and reduced menstrual bleeding; those experiencing either one or both of these (66% of subjects) were coded as having positive side effects of oral contraceptives. Experienced reduction of dysmenorrhea was calculated as an interaction term between having severe dysmenorrhea and experiencing positive side effects during the first 3 months of oral contraceptive use. Subjects were counted as experiencing a reduction in dysmenorrhea if they had both severe dysmenorrhea and experienced positive side effects (12.7% of subjects).

The analysis also reports associations between dysmenorrhea and other variables relevant to dysmenorrhea and oral contraceptive use. (These variables, including sociodemographic factors, sexual history, and health behaviors, are listed in Table III).

Results

The overall prevalence of dysmenorrhea in this population was 79.6%; 45.3% (141 subjects) reported mild dysmenorrhea, 15.6% (48) reported moderate dysmenorrhea, and 18.2% (56) reported severe dysmenorrhea (Table II). These figures are similar to those reported by Andresch and Milsom[2]; the overall prevalence is somewhat lower than that found in a study of 88 adolescent females.[9]

The relationship between subject characteristics and severity of dysmenorrhea are displayed in Table III. There were no significant differences by age, race, or mother's education. Previous literature suggested that parity (parous vs nonparous) would be negatively

Table IV. Multiple logistic regression analysis of predictors of consistent oral contraceptive use*

Predictor	*Regression coefficient*	*Standard error*	*Relative odds†*	*p Value*
Severe dysmenorrhea	−1.250	0.821	0.29	0.13
Experience one or more positive side effects	0.297	0.300	1.35	0.32
Severe dysmenorrhea and one or more positive side effects (interaction term)	2.080	0.903	8.04	0.02
Parous before baseline interview	−0.040	0.427	0.96	0.93
Started menstruation before age 12	−0.113	0.274	0.89	0.68
Smoke cigarettes at least once a month	−0.602	0.343	0.55	0.08
Intercourse once a week or more	0.463	0.273	1.59	0.09
Sometimes or always have painful intercourse	−0.368	0.293	0.69	0.21
Return visit to clinic	0.500	0.255	1.65	0.05

*All coefficients are adjusted for age, race, and mother's education.

†This is the antilog of the regression coefficient. It shows the expected change in odds of the outcome corresponding to a unit change in the predictor.

related to dysmenorrhea and that early onset of menstruation would be positively related to dysmenorrhea.[2, 10] Early onset of menstruation was marginally significant ($p \leq 0.07$); those who began menstruating before age 12 were somewhat more likely to have dysmenorrhea. Parity was the only subject characteristic that was significant ($p \leq 0.03$). Those who were parous before baseline were less likely to report any degree of dysmenorrhea.

Table III also presents information on the relationship of dysmenorrhea to contraceptive and sexual behaviors. In these bivariate analyses, dysmenorrhea was not significantly related to consistent oral contraceptive use or to experiencing less cramping or less bleeding during menstruation. It is of clinical interest that 30.4% of those with severe dysmenorrhea experienced neither of these positive side effects of oral contraceptives.

Also notable is that the severity of dysmenorrhea was significantly associated with sometimes or always experiencing dyspareunia. Experiencing painful intercourse at least some of the time was reported by 14.3% of those without dysmenorrhea, 27% of those with mild dysmenorrhea, 29.2% of those with moderate dysmenorrhea, and 37.5% of those with severe dysmenorrhea. Although intercourse appears to be painful for a significant percentage of women with a dysmenorrhea, the latter subjects are just as likely to report having frequent intercourse (once a week or more) and to report being somewhat or very happy about having intercourse.

Table III also presents the relationship of dysmenorrhea to a set of health behaviors. Those with any degree of dysmenorrhea are significantly more likely to return to the clinic where they initially received the pills over the 6-month follow-up period, although they are no more likely to call the clinic for advice during this time.

The relationship of dysmenorrhea to cigarette and alcohol use was also explored. On the basis of previous findings, it was hypothesized that there would be a positive relationship between dysmenorrhea and alcohol use[10] and a relationship, although the literature has been mixed on the direction, with cigarette use.[2, 3, 10] Although no significant association between alcohol use and severity of dysmenorrhea was found, there was a positive and marginally significant association with cigarette use ($p \leq 0.08$).

A multiple logistic regression analysis was used to examine the effects of severe dysmenorrhea, experiencing positive side effects of oral contraceptives, and an interaction term that represents experiencing a reduction of dysmenorrhea as a result of oral contraceptive use on consistent oral contraceptive use. The analysis controlled for variables that were potentially related to dysmenorrhea or oral contraceptive use. Three sociodemographic variables were included as covariates: age, race, and mother's education (a proxy for socioeconomic status). Previous research suggested that older adolescents, whites, and those with higher socioeconomic status are more compliant with contraception.[11-14]

The results of this analysis appear in Table IV. The most statistically and clinically significant predictor of consistent oral contraceptive use was experiencing the reduction of dysmenorrhea as a result of oral contraceptive use; those with severe dysmenorrhea who reported positive side effects of the pill were eight times as likely to be consistent oral contraceptive users as others ($p \leq 0.02$). The only other statistically significant predictor of oral contraceptive use was returning to the same clinic for oral contraceptives (1.65 times as likely, $p \leq 0.05$)

Two predictors, tobacco smoking and frequency of

intercourse, were marginally significant. Those who smoked cigarettes at least once a month were about half as likely (0.55, $p \leq 0.08$) to be consistent oral contraceptive users. Those who had intercourse at least once a week were 1.59 times more likely to be consistent oral contraceptive users ($p \leq 0.09$). Other studies have reported similar findings. Frequency of intercourse was thought to increase the perceived risk of pregnancy, and thus those with more frequent intercourse would be expected to be more consistent oral contraceptive users.[15-17]

Comment

This analysis demonstrates that an important predictor of consistent oral contraceptive use among adolescent women attending an inner-city family planning clinic was experiencing the alleviation of dysmenorrhea during the first 3 months of oral contraceptive use. Both this finding and the finding of a high prevalence of dysmenorrhea in the adolescent population underscore the importance of screening for dysmenorrhea all women who are seen at family planning clinics. This screen can be as straightforward as the three-question scale used in this analysis, with further probing if a woman responds positively to these questions.

Adolescents are often poorly informed about menstrual events. One study[18] reported finding substantial ignorance and misinformation among female adolescents regarding the causes and treatment of dysmenorrhea. Once it is determined that an adolescent suffers from dysmenorrhea, it is important to presensitize her to the potential positive side effects of oral contraceptives. Many clinics routinely stress the negative side effects of oral contraceptives without giving similar emphasis to the positive ones. Without adequate information, an adolescent woman with dysmenorrhea may not connect the relief of her symptoms to consistent oral contraceptive use, nor might she use oral contraceptives long enough to experience relief.

It is also important to follow up on women with dysmenorrhea to determine if they are experiencing alleviation of their symptoms. Fully 30.5% of the women with severe dysmenorrhea did not perceive experiencing any positive side effects of oral contraceptives. Having severe dysmenorrhea alone was not predictive of oral contraceptive use.

Consistency of care, as measured by a return visit to the clinic, is important in the provision of health care to adolescent women. It was significantly and positively related to both having dysmenorrhea at baseline and using oral contraceptives consistently over the follow-up period. This implies that clinics should encourage their clients to return to the same site for care.

This analysis also found several other health problems that were significantly related to dysmenorrhea. The prevalence of dyspareunia is so high in this population that it is important for clinicians to ask about dyspareunia and to provide information on how to alleviate it. It is apparent that reproductive health care for adolescents needs to include an open and thorough discussion about sexual functioning.

There was also a positive association between the severity of dysmenorrhea and smoking. Although the findings in the literature are mixed, one possibility is that adolescents with dysmenorrhea use cigarette smoking in an attempt to alleviate symptoms. If this is the case, it is vital that clinicians counsel these women on the health risks inherent in smoking and on alternate ways to relieve symptoms (such as consistent oral contraceptive or analgesic use).

Although this study had several findings relevant to understanding dysmenorrhea and oral contraceptive use in female adolescents, it does have some limitations which need to be noted. The major limitation is that the population consisted of female adolescents who were motivated to seek contraception; they may not be representative of all sexually active adolescents or of all adolescents with dysmenorrhea. Another limitation is that the subjects were all recruited in one family planning clinic and may not be representative of all adolescents who seek contraceptive services.

There have been few studies of the effect of dysmenorrhea on health behavior in female adolescents. A replication of this study is needed to confirm some of the findings and to further explore the relationship between dysmenorrhea, sexual health, and contraceptive use.

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Nonsurgical management of penetrating uterine trauma in pregnancy: A case report

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A woman at 30 weeks' gestation with stab wounds entering the uterus was managed nonsurgically by serial examinations and continuous fetal monitoring. Delivery occurred 4 weeks later with good maternal and fetal outcome. (AM J OBSTET GYNECOL 1992;166:583-4.)

Key words: Abdominal injuries, pregnancy, stab wounds

Penetrating abdominal trauma is an uncommon complication of pregnancy, with gunshot wounds much more common than stab wounds.[1] We report a case of multiple stab wounds to the abdomen of a pregnant woman, with documented penetration of the uterus, which was managed nonsurgically with a good outcome.

Case report

A 26-year-old Hispanic woman at 30 weeks of gestation in her first pregnancy was brought to the hospital by ambulance after her husband inflicted multiple stab wounds to the abdomen and upper extremities with a steak knife. On admission, the patient was hemodynamically stable and the fetal heart rate was 140 beats/min. Stab wounds to the hands, right upper arm, and abdomen were cleaned and dressed. Obstetric ultrasonography revealed a single fetus in a breech presentation, with an estimated fetal weight of 1928 gm and no evidence of fetal injury. The fetal head was directly below one of the wounds. There was a normal amniotic fluid volume, and the placenta was anterior with no evidence of retroplacental blood clot. Continuous fetal heart rate monitoring, serial hematocrit determinations, and serial abdominal examinations were undertaken. A Kleihauer-Betke test for fetal erythrocytes was negative. An amniocentesis was performed to rule out amnionitis. The amniotic fluid was grossly bloody, but Gram stain and subsequent culture did not reveal any organisms. The hematocrit was stable over 24 hours at 30%, the fetal heart rate was reactive without decelerations, and no signs of peritonitis developed. The patient was then observed in the hospital for an additional 48 hours. No signs of infection developed, and she was discharged from the hospital.

In the obstetrics clinic, there were normal heart tones and her wounds were healing well. At 34 weeks of

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gestation, she was admitted to the hospital in labor. The cervix was completely effaced and 4 cm dilated, and the fetus was in a transverse lie. Fetal heart rate monitoring was normal. External version to the vertex position was performed without difficulty. On rupture of the membranes, the fluid was noted to be dark brown with particulate matter present. The first stage of labor lasted 9½ hours and required oxytocin augmentation. The second stage of labor lasted 1 hour. A vigorous female infant weighing 2540 gm with Apgar scores of 8 at 1 minute and 8 at 5 minutes was delivered spontaneously. The uterus was explored manually and was intact.

The infant had a clinical estimation of gestational age of 35 weeks. The peripheral hematocrit at birth was 60% and was stable. A poorly healed laceration about 5 mm long that was noted over the infant's left parietal bone closed spontaneously on the second day of life.

Comment

Evaluation of the pregnant patient with either gunshot or stab wounds of the abdomen must take into consideration the physiologic changes of pregnancy while following the principles of trauma management. Pregnant women have an increased tolerance to blood loss because of their increased blood volume and are also less likely to have guarding and rigidity with peritoneal irritation. Although a patient with a gunshot wound generally requires surgical exploration, a patient with a stab wound to the abdomen may require exploratory laparotomy only if there is evidence of intraperitoneal hemorrhage or bowel perforation.[2] Cesarean section may be required if fetal distress ensues. The decision to deliver the fetus is frequently complicated by fetal prematurity and the difficulty in determining the extent and likelihood of repair of the fetal injury.

In this patient grossly bloody amniotic fluid suggested that the peritoneal cavity and uterus had been penetrated. Because there was no evidence of maternal or fetal hemorrhage or bowel injury, exploratory laparotomy was deferred. It seems unlikely, in view of the injury noted at delivery, that earlier delivery would have improved the outcome of the infant in any way. This case supports recommendations in the literature that injuries known to penetrate the uterus may be managed with the fetus left in utero if there is no evidence of fetal distress or amnionitis.

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Doppler ultrasonographic features of the developing placental circulation: Correlation with anatomic findings

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Color Doppler was used to study the placental circulations of 28 pregnancies between 10 and 17 weeks' gestation. A histomorphometric investigation was performed on placental tissue samples from those pregnancies, and the results were compared with Doppler features. Important changes were found around 14 weeks' gestation in both uterine and umbilical circulations. The appearance of end-diastolic frequencies in the umbilical arteries was related to an abrupt and significant ($p < 0.005$) increase in uterine velocity and to the establishment of a continuous intervillous circulation. (Am J Obstet Gynecol 1992;166:585-7.)

Key words: Doppler ultrasonography, placental circulation, early pregnancy

The beginning of fetal life is associated with remarkable hemodynamic changes in the placental circulations. During the first 3 months of gestation, the growing embryo is separated from the maternal circulation by the trophoblastic shell.[1] The tips of the spiral arteries are obstructed by intravascular trophoblastic plugs, and the intervillous space is bathed by a clear fluid, possibly made of filtered plasma and uterine gland secretions. The spiral arteries widen progressively, and around 12 weeks' gestation the trophoblastic plugs are eventually loosened and dislocated, allowing free blood circulation in the intervillous space.[1]

The purpose of this study was to investigate Doppler flow velocity waveform characteristics in the placental circulation between 10 and 17 weeks' gestation and to correlate these with anatomic features of placental vascular development.

Material and methods

Twenty-eight pregnant women between 10 and 17 weeks' gestation were investigated, four cases for each gestational week between 12 and 15 weeks and three for each of the other weeks. All were referred for ultrasonographic examination before termination of pregnancy for psychosocial reasons. Only patients who were certain of their last menstrual period, who were not using oral contraceptives, and who had never smoked were included in the study. All pregnancies were clinically uncomplicated, and ultrasonographic measurement of crown-rump length or biparietal diameter was within the normal range for gestational age.

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After giving informed consent, each woman underwent Doppler examination with 5.0 MHz curvilinear transvaginal probe with pulsed and color Doppler facilities (Aloka Color Doppler SSD-680, Aloka Co., Tokyo). The high-pass filter was set on 100 Hz and the pulse repetition frequency was 2 to 12 kHz. Color Doppler was used to identify vessels of interest and served as a guide for pulsed Doppler velocimetry studies. In each case flow velocity waveforms were obtained from both main uterine arteries at the level of the internal os, from spiral arteries at the level of the placental bed, and from umbilical cord arteries. The intraplacental areas of color flow were also investigated by spectral Doppler analysis. Simultaneous color imaging made it possible to obtain the maximum peak velocity in the uterine artery by positioning the transducer so that the angle between the pulsed Doppler beam and the vessel was close to zero. Uterine flow velocity waveforms were standardized by means of the resistance index, calculated as the mean obtained from three waveforms of satisfactory quality and averaged for both sides.

Terminations of pregnancy were performed within 2 hours after Doppler ultrasonographic investigations. In each case samples of placental tissue were obtained by aspiration and were immediately immersed in formalin. After 3 days of fixation the specimens were embedded in paraffin, sectioned at 4 μm intervals, and stained with hematoxylin and eosin for histologic examination. The sections were viewed with an immersion objective, connected to a video system. Twenty normally trophic, stem villi were examined at random for each case. A point-counting grid was superimposed onto the image, and the fraction of the villous surface (percent) occupied by the fetal vessels was estimated by test point counting. The number of vessel profiles per villous profile was also calculated.

The relationships between gestational age and Doppler and histologic data were analyzed by simple regres-

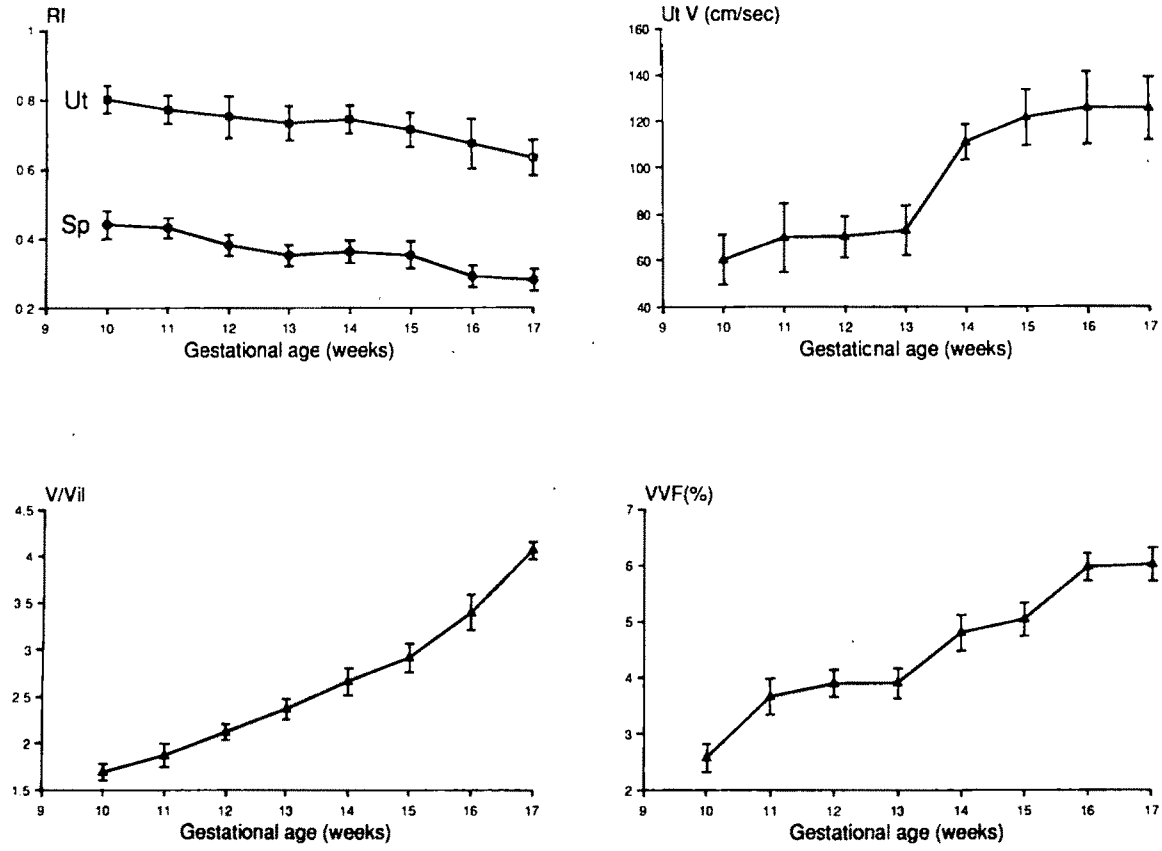


Fig. 1. Mean values (±SEM) per week for uterine *(Ut)* and spiral *(Sp)* resistance index *(RI)*, uterine peak velocity *(UtV)*, number of vessels per villous profile *(V/Vil)*, and vascular volume fraction *(VVF)*.

sion models. Where appropriate, significant differences were calculated by the Kruskal-Wallis rank test.

Results

The main uterine, spiral, and umbilical cord arteries were visualized by color Doppler in all cases. The best regression fit between gestational age and each parameter was observed to be linear for uterine resistance index ($r = -0.38$, $n = 28$, $p < 0.05$, $a = 0.96$, $b = -0.02$, SEM = 0.09) and spiral resistance index ($r = -0.61$, $n = 28$, $p < 0.001$, $a = 0.61$, $b = 0.02$, SEM = 0.05) and exponential for uterine peak systolic velocity ($r = 0.74$, $n = 28$, $p < 0.001$, $a = 2.86$, $b = 0.12$, SEM = 0.25), number of vessel profiles per villous profile ($r = 0.93$, $n = 28$, $p < 0.001$, $a = -0.66$, $b = 0.12$, SEM = 0.1), and vascular villous fraction ($r = 0.84$, $n = 28$, $p < 0.001$, $a = 0.03$, $b = 0.1$, SEM = 0.16).

Fig. 1 shows the results expressed as a mean (±SEM) for week of gestational age for each parameter. Table I summarizes the measurements. Uterine and spiral artery waveforms showed a smooth progressive decrease of resistance index values with gestation. This change was significant before 13 ($p < 0.01$) and after 14 ($p < 0.05$) weeks of gestation for the spiral resistance index only. The mean peak systolic velocity in the uterine artery increased slowly but not significantly with gestation from 10 to 13 weeks. However, during the fourteenth week an abrupt and significant ($p < 0.005$) increase in peak systolic velocity was observed. After that, a slow increase continued until 17 weeks (Fig. 1).

Between 12 and 14 weeks, end-diastolic frequencies in the umbilical artery were incomplete or inconsistently present. At the same time, a nonpulsatile blood flow pattern was detected when the Doppler gate was placed on intraplacental sonolucent spaces. Pandiastolic frequencies in the umbilical artery and continuous venous flow within the placenta were consistently present in all cases later in gestation. The number of vessel profiles per villous profile and the vascular villous fraction increased significantly with gestation (Table I).

Comment

The current study shows that major hemodynamic changes occur in both fetal and maternal placental circulations around 14 weeks of gestation. At that time we observed blood flow with a venous pattern, i.e., intervillous flow within the placenta, an abrupt and sig-

Table I. Mean values (±SEM) obtained for resistance index in uterine and spiral arteries, for uterine peak systolic velocity, for number of vessels per villous profile, and for villous volume fraction occupied by fetal vessels

	Duration of gestation			
	10-11 wk (n = 6)	*12-13 wk (n = 8)*	*14-15 wk (n = 8)*	*16-17 wk (n = 6)*
Uterine resistance index	0.77 ± 0.04	0.76 ± 0.03*	0.70 ± 0.04*	0.69 ± 0.41*
Uterine velocity	67.55 ± 8.49	73.60 ± 6.49*	117.39 ± 7.04†	127.09 ± 9.29*
Spiral resistance index	0.44 ± 0.19	0.37 ± 0.09‡	0.36 ± 0.27*	0.31 ± 0.16§
Vessels per villous profile	1.80 ± 0.12	2.25 ± 0.08§	2.79 ± 0.87†	3.74 ± 0.17†
Villous volume fraction	3.16 ± 0.33	3.89 ± 0.20§	4.96 ± 0.27‡	5.98 ± 0.05§

*Not significant.
†$p < 0.005$.
‡$p < 0.01$.
§$p < 0.05$.

nificant increase of the mean uterine peak systolic velocity (Fig. 1), and the appearance of pandiastolic frequencies in the umbilical arteries.

Theoretically, the resistance to blood flow and the variation in the shape of the flow velocity waveforms reflect changes in the resistance of the distal vascular bed. Previous authors have shown that the appearance of diastolic frequencies in the umbilical arteries of early pregnancy is associated with an abrupt fall of the resistance index in the umbilical artery.[2] We believe that these changes do not reflect a sudden opening of the villous capillaries or a merging between villous and embryonic vessels, as suggested by these authors. Placental angiogenesis starts during the fifth week after the last menstruation, and all specimens that we have investigated had well-formed villous vessels 4 weeks before the appearance of diastolic frequencies. Their number and the surface of stem villi occupied by these vessels increased significantly between 10 and 17 weeks' gestation. Although these progressive anatomic changes in the villous vasculature must have an important role in the gradual fall in the resistance to blood flow in the umbilical circulation, the appearance of end-diastolic frequencies coincided with a significantly higher blood velocity in the main uterine arteries and with the presence of a continuous intervillous flow. The establishment of the intervillous circulation at the beginning of the second trimester of pregnancy may also be associated with changes in the pressure gradient between the uterine circulation and the intervillous space or with alterations in blood gases and metabolites, which could influence the specific properties of the placental vessels and therefore the appearance of end-diastolic frequencies in the umbilical circulation. Longitudinal histomorphometric study of the villous vasculature would be unethical and focal villous sampling would not give a representative picture of the placenta as a whole. However, serial Doppler ultrasonography can be performed to establish more accurately the close relationship that exists between the development of the two placental circulations in early pregnancy.

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Routine urine drug screening at the first prenatal visit

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OBJECTIVE: The purpose of this study is to determine whether routine urine drug screening at the first prenatal visit will identify and permit early intervention for those at risk for poor outcome.

STUDY DESIGN: Comparisons of maternal and neonatal outcomes between 166 randomly chosen patients with positive and 150 randomly chosen patients with negative drug screens identified by the enzyme multiplied immunoassay test were made by unpaired t test, χ^2 test, probit analysis, and tests of independent Poisson distributions.

RESULTS: Forty percent of those identified denied drug use. Antepartum ($p < 0.01$) and postpartum ($p < 0.05$) complications were increased among drug users. Birth weight ($p < 0.001$), gestational age ($p < 0.03$), and head circumference ($p < 0.05$) were decreased among neonates.

CONCLUSION: Because of difficulty in identifying illicit substance–using patients, consideration should be given to the implementation of routine urine drug screening at the first prenatal visit in populations with a high rate of illicit substance use. (Am J Obstet Gynecol 1992;166:588-90.)

Key words: Drug screening, prenatal care, substance abuse

Complications from illicit drug use during pregnancy have increased at an alarming rate. Substance abuse has been associated with low birth weight, premature deliveries, and sexually transmitted diseases.[1, 2] Since 1985 we have noticed an increase in illicit substance use among pregnant women registered for prenatal care through the Outpatient Services Department of the Wilmington Hospital of the Medical Center of Delaware. To identify patients at risk for complications of pregnancy associated with substance abuse, we began performing routine urine drug screening at the first prenatal visit. We offer counseling and special services to patients with urine drug screens positive for illicit drug use in an effort to decrease the potential risks to mother and child.

The purpose of this study was to evaluate the role of urine drug screening as a component of routine prenatal care. Specifically, we sought to determine if urine drug screening is an effective method of identifying those women who will benefit from drug counseling and supportive services.

Material and methods

Although a careful history is performed by both nurses and physicians at the first prenatal visit, self-reporting of drug use is of limited accuracy. Thus all patients registered for prenatal care through the Outpatient Services Department (teaching service) of the Wilmington Hospital routinely submit a urine sample for screening by the enzyme multiplied immunoassay test to determine the presence of cannabinoids, cocaine, opiates, barbiturates, amphetamines, and benzodiazepines. Positive results are confirmed by means of thin-layer chromatography, gas chromatography, and gas chromatography–mass spectrophotometry as appropriate for the presence of specific substances. Patients are informed of drug testing by a pamphlet that describes laboratory procedures performed during pregnancy.

From July 1, 1988, through June 30, 1989, approximately 6700 women were delivered at the Medical Center of Delaware. Of these, 1088 women registered through the Outpatient Services Department (teaching service) were delivered. Those with a positive drug screen ($n = 166$) were compared with a sample of 150 patients selected by means of a random number table from the remainder of the population for whom the initial drug screen was negative. Patients who admitted alcohol use were screened by serum testing.

We compared outcome criteria (preterm labor, bleeding during pregnancy, premature rupture of the membranes, hepatitis, human immunodeficiency virus infection, delivery method, gestational age at delivery, twinning, fetal monitor problems, antepartum and postpartum complications, birth weight, Apgar scores, malformations, head circumference, neonatal withdrawal, neonatal seizures, neonatal tachycardia, neonatal hyperirritability, intraventricular hemorrhage, patent ductus arteriosus, necrotizing enterocolitis, meconium staining, and need for neonatal mechanical ventilation) for substance users versus controls. Unpaired t test, χ^2 test, probit analysis, and tests of in-

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Table I. Antepartum complications

Complication	*Controls (n = 150)*	*Drug users (n = 166)*
Abruptio placentae	3	15
Anemia	2	13
Appendicitis	1	0
Asthma	0	5
Cervical dysplasia	1	5
Depression	0	2
Gestational diabetes	2	1
Insulin-dependent diabetes	1	1
Human immunodeficiency virus infection	0	6
Hypertension, chronic	1	2
Hypertension, pregnancy-induced	6	4
Hyperthyroidism	0	1
Lupus erythematosus	0	1
Nephrolithiasis	0	2
Nephrotic syndrome	0	1
Ovarian cyst	0	1
Pituitary adenoma	1	0
Pneumonia	0	4
Preterm labor	22	55
Premature rupture of membranes	8	33
Pyelonephritis	1	4
Seizure disorder	0	2
Sexually transmitted disease	8	14
Thrombophlebitis	2	1
Urinary tract infection	5	5
TOTAL	64*	178*

*$p < 0.05$.

Table II. Postpartum complications

Complication	*Controls (n = 150)*	*Drug users (n = 166)*
Anemia	2	13
Endometritis	2	6
Disseminated intravascular coagulation	0	1
Hypertension, pregnancy-induced	0	3
Pneumonia	0	2
Spinal headache	2	1
Urinary tract infection	0	1
Wound dehiscence	0	2
TOTAL	6*	29*

*$p < 0.05$.

dependent Poisson distributions were used for inference.

Results

The substance abusing and control populations were compared with regard to several demographic factors to determine differences between the groups. No significant difference between the study and control populations was found with regard to racial composition (50% black, 45% white, 5% Hispanic [both groups exactly the same]), cigarette use (39% vs 45%), or parity (substance abusers' mean parity = 1.3 vs controls' mean parity of 1.0). A statistical difference was found with regard to age (substance abusers' mean age was 23.9 years vs controls' mean age of 22.1 years [$p < 0.05$]).

The incidence of abuse of one or more drugs was found to be 15.3% among the Outpatient Services Department patients. The drugs used by this population were cocaine ($n = 104$, 62%), marijuana ($n = 100$, 60%), alcohol ($n = 22$, 13%), amphetamines ($n = 10$, 6%), opiates ($n = 10$, 6%), and barbiturates ($n = 6$, 3%). Among substance users, 90 (54%) admitted drug use at the first prenatal visit, 66 (40%) denied drug use, and data for 10 (6%) were not available. The rate of denial of illicit drug use among those women who eventually stopped using drugs (39%) was not significantly different from that of those who continued (41%).

Among maternal outcome criteria, antepartum complications (Table I) and postpartum complications (Table II) were found to be significant ($p < 0.01$ and $p < 0.05$, respectively) when controls were compared with substance users.

Of neonatal outcome criteria, a significant difference between the controls and substance abusers was found for birth weight, gestational age, and head circumference data (Table III). We found no significant differences between the groups with regard to the other outcome criteria studied.

Comment

Among those women in our population who use drugs, we found that approximately 40% denied drug use at the first prenatal visit. Interestingly, this was equally true for the subpopulations that discontinued (39%) and continued (41%) drug use. The failure of patients to report behavior that is inimical to the health of themselves and their fetuses is likely due to complex factors, including social stigma, fear of legal prosecution, and an understanding that they might be coerced into accepting a medical treatment program. Although the population studied is indigent, it is possible that a survey of the private population served by our hospital would have yielded similar results. Chasnoff et al.,[3] in their recent study of substance use in private and clinic populations, have found an incidence of 14.8% among pregnant women, a rate similar to that found in our population. They found no significant difference in the rate of illicit substance use between private and clinic populations. This high rate of substance use in pregnancy places many mothers and fetuses at risk for adverse pregnancy outcomes.

Several authors have reported an increased rate of

Table III. Neonatal outcome

Outcome	*Controls (n = 150)*	*Drug users (n = 166)*	*p*
Birth weight (gm)	3255 ± 632	2905 ± 738	<0.001
Gestational age (wk)	39.0 ± 2.3	37.3 ± 3.5	<0.003
Head circumference (cm)	34.0 ± 1.9	32.7 ± 2.8	<0.05

anemia,[4] preterm labor,[1, 5, 6] abruptio placentae,[6, 7] premature rupture of the membranes,[5] and premature delivery[1, 5, 7, 8] among substance users, especially those who use cocaine. We also found an increased risk of antepartum and postpartum complications when comparing drug users with controls (Tables I and II, respectively).

An increased risk of fetal monitoring abnormalities, meconium staining, and anemia also have been reported as complications of maternal illicit substance use.[4] We found no differences with regard to these parameters between controls and the substance users.

Low birth weight, earlier gestational age at delivery, and smaller head circumference have been described as complications of cocaine[1, 2, 4, 5, 7, 8] and heroin[9] use. Several authors have reported an increased incidence of anomalies among the offspring of pregnant substance users.[1, 4] The rate of anomalies among cocaine abusers has been reported as 5.7%, versus 1.4% among controls.[1] Genitourinary tract[2] and cardiac anomalies[4] have been reported in the infants of these patients. Whether the increase in the rate of anomalies among the infants of substance users is due to exposure to illicit substances or to other factors in the life-styles of their mothers, such as poor nutrition, is unclear.[4] When comparing the neonates of drug users with those of controls in our population, we found no statistical difference in the rate of anomalies.

A key issue in the management of illicit substance use in pregnancy is the identification of the problem. Others have demonstrated that management of these patients in a supportive program can be successful. However, the dangers of our approach to the identification of the patient must be acknowledged. This approach could not be advocated in those states that take a punitive approach to the problems of pregnant drug users. The great danger of routine drug testing is that the state may subpoena this medical information for use in the prosecution of the patient. Obstetrician-gynecologists and their staffs should not be obligated to function as agents for the state.[10]

In some populations illicit drug use may not be as widespread, and routine drug testing may not prove to be a cost-effective method of screening for drug use. Thus we recommend that a blinded survey of the population to be screened be performed before the implementation of routine urine drug testing. Potential problems resulting from drug screening include late entry into prenatal care and the failure to identify those drug using patients who abstain from drug use before their first prenatal visit and resume or begin drug use afterward.

This study found that approximately 40% of pregnant substance users denied drug use. Routine drug screening permitted the identification of these patients. Given the prevalence of maternal illicit substance use in populations such as ours, we propose that consideration be given to the establishment of urine drug screening at the time of the first prenatal visit as a component of routine prenatal care. We recognize that in some populations the knowledge that drug screening is being performed may serve as a deterrent for patients to enter prenatal care. However, if the care provided is nonjudgmental and supportive, we feel that drug screening provides a useful adjunct in the management of pregnancies at high risk for exposure to illicit drugs.

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Bilateral choroid plexus cysts in trisomy 21

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Whether karyotyping is indicated in a fetus with choroid plexus cysts who is otherwise structurally normal is still controversial. Many authors have suggested basing the decision on cyst size, bilaterality, persistence with advancing gestational age, and association with other anomalies. We report a case of large bilateral choroid plexus cysts in a fetus with trisomy 21 who had no evidence of congenital anomalies or ultrasonographic signs of Down syndrome. Cyst sizes diminished by half over a 3-week period of follow-up. It appears that diminishing size alone should not be considered sufficient reassurance about the normality of the fetal karyotype. A similar case has been previously reported, and it is conceivable that choroid plexus cysts are associated not only with trisomy 18 but also with trisomy 21. (AM J OBSTET GYNECOL 1992;166:591-2.)

Key words: Choroid plexus cyst, trisomy 21

The prenatal ultrasonographic finding of choroid plexus cysts has been described in fetuses with trisomy 18, as well as in those with normal chromosomes. Whether karyotyping is indicated in a fetus who otherwise appears to be structurally normal has been the subject of much recent debate. Only one case of antenatally diagnosed choroid plexus cysts in a fetus with trisomy 21 has been reported in the literature.[1] This fetus also had an atrioventricular septal defect. We present a case of trisomy 21 in which the only ultrasonographically apparent abnormalities were bilateral choroid plexus cysts.

Case report

A 35-year-old woman, gravida 4, para 3, presented for routine ultrasonographic examination at 16 weeks' gestation, before amniocentesis for karyotyping. Biometric measurements were consistent with gestational age. Gross fetal anatomy appeared normal, except for the presence of bilateral choroid plexus cysts of 10 mm diameter in each atrium of the cerebral ventricles. The four-chamber view of the heart showed no abnormality. The nuchal fold thickness measured 3 mm. The middle phalanx of the fifth digit was equal in size to the middle phalanx of the fourth finger. The expected-to-actual femur length ratio and the biparietal diameter/femur length ratio were within the mean. The maternal serum α-fetoprotein level was within normal limits. An amniocentesis was performed and 16 ml of clear fluid was obtained. The karyotype was 47,XX +21.

The patient was counseled about the implications of the karyotype and decided to terminate the pregnancy at 19 weeks' gestation by dilation and evacuation. A repeat ultrasonographic scan before termination reconfirmed normal fetal anatomy and bilateral choroid plexus cysts, each of which had shrunk to 5 mm in diameter.

Comment

Cysts of the choroid plexus arise from neuroepithelial folds within the choroid plexus and are filled with fluid and cellular debris. They are found frequently on microscopic examination of fetal, newborn, and adult brains. The incidence of macroscopically apparent cysts in chromosomally normal subjects, however, has probably been overestimated because of the use of histologic criteria for the definition of their presence. The estimated incidence of ultrasonographically diagnosed choroid plexus cysts in a population of normal midtrimester fetuses is 0.3%. An association with trisomy 18 has been suggested[2]; however, the magnitude of this link is controversial.

Choroid plexus cysts associated with trisomy 21 have so far been described in the literature in only one case.[1] This fetus also had an atrioventricular septal defect. In our case bilateral choroid plexus cysts were the only abnormal finding in a detailed anatomic survey. Whether the finding was incidental or linked to the chromosomal anomaly is difficult to determine. If such an association is assumed, it is probably not a very consistent one, because multiple series of fetuses with Down syndrome have not reported this finding. However, a review of 98 prenatally diagnosed cases of trisomy 21 in our institution revealed two such cases. Unfortunately, only one case had complete ultrasono-

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graphic documentation. Even if the association between cysts of the choroid plexus and trisomy 21 was 1 in 100, most women would still wish to be offered prenatal diagnosis. However, it would require 120,000 midtrimester ultrasonographic scans to demonstrate a prevalence of this magnitude within 95% confidence limits of 0.5% and 1.5%.

It is intriguing that the chorcid plexus cysts in our case displayed multiple characteristics that were associated with a trisomic karyotype by several authors. Chitkara et al.[2] suggested that a cyst diameter >10 mm and a bilateral location, as observed in our case, are more likely associated with a trisomic fetus. This tends to suggest that the finding of choroid plexus cysts in this case was not incidental but was linked to the abnormal karyotype. Interestingly, we observed a 50% decrease in cyst diameter within a 3-week period of follow-up. Therefore diminishing cyst size alone should not be considered sufficient reassurance about the normality of the fetal karyotype. Furthermore, this finding emphasizes that in spite of improved ultrasonographic visualization of anatomy with advancing gestational age early scans can occasionally contribute exclusive information.

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Radical hysterectomy in the elderly patient: Analysis of morbidity

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OBJECTIVE: This study compares the perioperative morbidity and mortality following radical hysterectomy of patients older than 65 years with a younger age group who underwent radical hysterectomy and pelvic lymphadenectomy for cervical carcinoma stage IB or IIA.

STUDY DESIGN: A retrospective analysis of morbidity and mortality for the first 60 postoperative days was conducted. The study population of 45 women >65 years of age with cervical cancer treated by radical hysterectomy was compared with a control population of 90 women <65 years treated similarly.

RESULTS: In the elderly group, 31 of 45 and 12 of 15 were American Society of Anesthesiologists Physical Status II and III respectively; 68/90 and 19/90 were American Society of Anesthesiologists status I and II in the younger age group ($p = 0.001$). Transfusions of >2 units were required in 14% of the elderly and 35% of younger patients ($p = 0.02$). No statistical differences were observed between groups for other parameters examined.

CONCLUSION: Age alone should not be a contraindication for radical hysterectomy in the elderly patient with American Society of Anesthesiologists Physical Status I to III. (Am J Obstet Gynecol 1992;166:593-7.)

Key words: Geriatric, radical hysterectomy, cervix carcinoma

The controversy between surgical treatment, radical hysterectomy with bilateral pelvic lymphadenectomy, and radiotherapy in the treatment of women with stage IB or IIA cervical carcinoma has existed for decades. Retrospective series of both types of treatments show no significant difference in survival for either regimen.[1] Surgical therapy is preferred in younger women because of the preservation of hormonal function and avoidance of the radiotherapy-related sexual dysfunction.[2]

The advantage of radiotherapy is its applicability to virtually all patients, whereas radical surgery excludes certain medically inoperable cases. Traditionally, operation has been considered to be contraindicated in older women because of unacceptable increases in morbidity and mortality thought to be associated with advanced age and related concomitant disease.

Over the last two decades significant advancements have been made in the medical management of patients undergoing radical surgery. The purpose of this study was to compare the intraoperative and postoperative morbidity and mortality of patients older than 65 years undergoing radical hysterectomy to younger patients undergoing similar treatment.

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6/1/32388

Material and methods

The study (elderly) population consisted of 45 patients aged ≥65 years who were diagnosed with cervical cancer, stage IB or IIA, and treated with radical hysterectomy and pelvic lymphadenectomy by the Division of Gynecologic Oncology at the University of California, Irvine Medical Center and Long Beach Memorial Medical Center from January 1980 through December 1989. The control (younger) population consisted of the next two patients younger than 65 years with a diagnosis of cancer of the cervix who were admitted for the same operation after a study case admission. Controls were matched to study cases by diagnosis, stage, and type of operation. Cases were identified by the respective institutional tumor registries. Medical records were reviewed for both groups and analyzed for morbidity and mortality for the first 60 postoperative days. Follow-up information was obtained from the tumor registry, patient records, referring physicians, and death certificates.

All patients underwent a radical hysterectomy, Meigs

Table I. Characteristics of study population

Characteristic	Elderly (n = 45)		Younger (n = 90)		Significance
	No.	%	No.	%	
Stage IB*	41	91.1	79	87.8	NS
Stage IIA*	4	8.9	11	12.2	NS
Squamous cell carcinoma	35	77.8	71	78.8	NS
Occult parametrial disease	4	8.9	9	10.0	NS
Lymph node metastasis	12	26.6	32	35.6	NS
Bilateral lymph node metastasis	5	11.1	14	15.6	NS
Lesion size >30 mm	14/35	40.0	50/84	59.5	NS
Poorly differentiated disease	11/42	26.2	42/82	51.2	$p = 0.008$

NS, Not significant.
*International Federation of Gynecology and Obstetrics.

Table II. American Society of Anesthesiologists physical status

American Society of Anesthesiologists status	Elderly		Younger	
	No.	%	No.	%
I	1	2.2	68	75.6
II	31	68.9	19	21.1
III	12	26.7	3	3.3
IV	1	2.2	—	—
V	—	—	—	—
TOTAL	45	100	90	100

Kolmogorov-Smirnov two-sample test, $p = 0.001$.

type 3,[3] and pelvic lymphadenectomy as described by Taussig.[4] Prophylactic antibiotics were used in all cases. The majority of patients received cephalosporin for 24 hours. Prophylactic heparin, 5000 IU given subcutaneously every 12 hours, was used in all patients until 1984. Subsequent patients were treated with pneumatic calf compression. Drains were routinely used and were positioned on the pelvic sidewalls with the exit through stab wound incisions. We used the American Society of Anesthesiologists physical status classification system[5] in determining the potential surgical risks. Surgical infection morbidity was analyzed on the basis of clinical evaluations and cultures and classified according to a modification of the criteria used by Sevin et al.[6] Surgical site–related infection consisted of: (1) sepsis, (2) pelvic abscess or hematoma associated with fever, (3) pelvic cellulitis, (4) wound infection with purulent wound breakdown, or (5) other intraabdominal infections distant from the operative site. Non–surgical site–related infection included: (1) urinary tract infection or (2) postoperative respiratory infection. Morbidity not related to infection included: (1) fever of 38.0° to 39.4° C orally within the first 48 hours (e.g., pulmonary atelectasis), (2) fever of 38.0° C orally on two separate postoperative occasions at least 6 hours apart, >48 hours after the end of the procedure (e.g., no infection identifiable), or (3) sterile wound separation with negative cultures.

Statistical analysis was performed with Pearson χ^2, Student *t* test, and Kolmogorov-Smirnov analysis of categoric data.[7] Life-table analysis was performed with the Kaplan-Meier method[8] and survival functions were compared with the Mantel-Cox model.[9]

Results

The study group consisted of 45 patients, with ages ranging from 65 to 83 years (mean 70.1 ± 4.2), with 15 (33.3%) patients aged ≥70. The 90 patients <65 years old included in the control group ranged in age from 19 to 64 years, (mean 41.7 ± 10.9). The histologic characteristics and stages for the study population and younger control group demonstrate no statistical difference in stage, histologic type, tumor volume, frequency of lymph node metastasis, and the finding of occult parametrial disease (Table I). Poorly differentiated tumors were significantly less frequent in the elderly patient group ($p = 0.008$). A significant difference was found in the anesthetic risk of the study versus control groups. In the geriatric population 31 (68.9%) and 12 (26.7%) patients were American Society of Anesthesiologists class II and class III, respectively, whereas in the younger group 75.8% and 20.9% were class I and class II, respectively ($p = 0.001$) (Table II). Of the elderly patients, 10 had no known previous medical problems, whereas the remaining 35 patients had one or more preexisting medical illnesses. Only 19 of the 90 younger control patients presented with preexisting medical illnesses, with hypertensive ($p < 0.001$) and respiratory ($p = 0.003$) problems being the most significant (Table III).

Pelvic lymphadenectomies were performed in all cases. The number of nodes sampled ranged from 18.5 ± 8.1 in the elderly group to 21.4 ± 8.5 in the younger patients ($p > 0.1$). Paraaortic lymph nodes were sampled in 8 (17.8%) and 25 (27.5%) patients from the elderly and younger groups, respectively ($p = 0.215$).

The mean estimated blood loss during the surgery was 1150 ± 674 ml (range 300 to 3300) in the elderly group and 1513 ± 1596 ml (range 350 to 12,100) in

Table III. Preexisting illnesses in the study populations

	Elderly	*Younger*	*Significance*
Cardiovascular			
Hypertension (moderate or severe)	21	8	$p < 0.001$
Post–myocardial infarction or angina	2	0	$p = 0.044$
History of congestive heart disease	4	1	$p = 0.024$
Heart valve replacement or coronary bypass	2	1	NS
Coarctation of aorta or mitral valve prolapse	2	0	$p = 0.044$
Other	3	0	$p = 0.013$
Metabolic or endocrine			
Diabetes	4	1	$p = 0.024$
Hypothyroidism	3	0	$p = 0.013$
Obesity (severe)	1	4	NS
Malnutrition (severe)	1	0	NS
Respiratory			
Chronic obstructive disease of airway (moderate or severe)	7	2	$p = 0.003$
Other illnesses	7	4	$p = 0.026$
Patients with no preexisting illnesses	10	71	
Total No. of illnesses per total No. of patients	57/45*	21/90*	

NS, Not significant.
*Some patients presented with more than one illness.

the younger ($p > 0.1$). The high blood loss value of 12,100 ml represents one patient with trauma and hemorrhage to the hypogastric vein. More than two units of blood was required in 6 (13.6%) of the elderly group versus 29 (32.6%) in the younger group ($p = 0.020$). The duration of the operation was 4 hours 15 minutes in the elderly group and 4 hours 32 minutes ± 52 minutes in the younger groups ($p = 0.100$).

The incidence of surgery-related complications was 5 (11.1%) in the elderly group and 9 (9.9%) in the younger group ($p = 0.825$) (Table IV). These included two intraoperative ureteral injuries necessitating repair (0 elderly, 2 younger), 2 bladder or bowel injuries (1 elderly, 1 younger), and 7 vascular injuries (3 elderly, 4 younger). Three cases required postoperative surgical correction, 1 with bowel obstruction in each group and 1 with ureterovaginal fistula in the younger group.

Early postoperative medical complications occurred more frequently in the elderly group (11.1%) than in the younger group (5.6%) ($p = 0.238$) (Table V). In the elderly group one patient with severe chronic hypoventilation required ventilation assistance and was extubated after bronchodilator treatment on the second postoperative day. In the younger group a lateral pontomedullary infarct developed after a vertebral artery thrombosis in one patient and another patient required ventilation assistance because of fluid overload.

The overall incidence of infectious morbidity was similar in the elderly and the younger groups, 37.8% and 38.5%, respectively ($p = 0.938$) (Table VI). The incidence of surgical site infection and non–surgical site–related infection was 11.1% and 15.5% in the elderly group and 10.0% and 16.5% in the younger group.

Table IV. Surgical complications

	Elderly		*Younger*	
	No.	*%*	*No.*	*%*
Intraoperative				
Ureteral injury	0	0	2	2.2
Bladder or bowel injury	1	2.2	1	1.1
Artery vein laceration	3	6.7	4	4.4
Postoperative				
Bladder or ureteral fistula	0	0	1	1.1*
Intestinal obstruction	1	2.2*	1	1.1*
TOTAL	5/45	11.1†	9/90	10.0†

*Required surgical correction.
†Difference not significant.

Table V. Perioperative medical complications

	Elderly	*Younger*
Required ventilation assistance	1	1
Intraoperative hypotension (mild)	1	0
Premature atrial or ventricular contraction	1	1
Pelvic vein thrombosis	0	1
Cerebral vascular accident	0	1
Hypertensive crisis	2	1
TOTAL	5/45 (11.1%)*	5/90 (5.6%)*

*Difference not significant.

Five-year cancer-specific survival estimates were 89% and 79% in the elderly group and younger group, respectively ($p > 0.300$) (Fig. 1). Two deaths from undetermined causes were assumed to be cancer-related deaths.

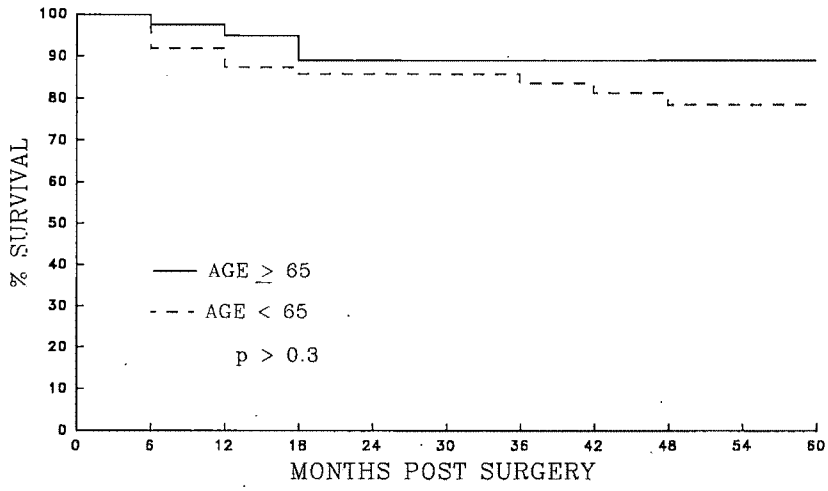


Fig. 1. Cancer-specific-survival for patients with stage IB or IIA cervical carcinoma managed with radical hysterectomy.

Table VI. Surgically related infection morbidity

	Elderly (n = 45)	*Younger (n = 90)*	*Significance*
Surgical site–related			
Septicemia	2		1
Pelvic cellulitis	1		0
Abcess	0		3
Wound infection	2		5
Subtotal	5 (11.1%)	9 (10.0%)	NS
Non–surgical site–related			
Pneumonia	2		0
Urinary tract	5		15
Subtotal	7 (15.5%)	15 (16.7%)	NS
Morbidity not related to infection			
Fever	5		7
Sterile wound separation	4		5
Subtotal	9 (20.0%)	12 (13.3%)	NS
TOTAL	21/17* (37.8%)	36/34* (37.7%)	NS

NS, Not significant.
*Number of complications per number of patients.

Comment

Radiotherapy was the accepted treatment for cervical cancer from the mid-1920s to 1945. Meigs[10] reintroduced the concept of radical surgery for treatment of cervical cancer in 1945. Over the following decades the perioperative morbidity and mortality decreased to very acceptable levels. Recently, because of advances in anesthesia, with improved intraoperative and postoperative monitoring, and the availability of effective broad-spectrum antibiotics, older patients are considered eligible for radical surgery that might have been previously denied.

In this study we have found the mortality and morbidity associated with radical hysterectomy to be similar in elderly and younger patient groups. The major morbidity found in the older group in our study is similar to the overall morbidity reported by others.[11-13] Kinney et al.[14] reported an 11% incidence of complications that required surgical correction in the geriatric population and an 8% incidence of pulmonary embolism. Our report compares very favorably, with only 2.2% of patients requiring reoperation and no cases of pulmonary embolism. The management of stage I cervical carcinoma with radiation therapy in the national practice has a major complication rate of 11%; this rate decreased to 5% in patients >65 years old but increased to 22% in patients <36 years old.[15]

Radical surgery is associated with a high rate of infection-related morbidity because of the extensive dead space created by the dissection, bacterial contamination from the vagina and the often lengthy duration of the operation. The surgical site–related infection rate of

11.1% is similar to the rate (15%) reported by others.[6] The total infection-related morbidity incidence of 38% is within the 23% to 52% range previously reported.[6, 16]

Radical hysterectomy is an operation that traditionally has been reserved for use in the younger population. Our study shows no difference in perioperative complications of radical hysterectomy between younger and older groups. Furthermore, it demonstrates that this is a safe operation that can be performed in elderly patients with American Society of Anesthesiologists physical status I to III.

In summary, with modern perioperative management we are now able to offer this operation to older patients. Age alone should not be considered a contraindication to radical hysterectomy.

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Postpartum paravaginal hematoma and lower-extremity infection

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We report a case of infection of the lower extremity after a normal vaginal delivery. The infection originated in an occult obturator internus muscle hematoma, and diagnosis was based on a clinical suspicion and characteristic findings on computerized tomography scan. These findings permitted prompt surgical drainage, debridement, and antibiotic therapy and resulted in a successful outcome. (AM J OBSTET GYNECOL 1992;166:598-600.)

Key words: Paravaginal hematoma, necrotizing fascitis

Infection of the lower extremity is an extremely rare condition in obstetric patients Retropsoal and subgluteal infections and necrotizing fascitis of the lower extremity have all been reported[1, 2] in the obstetric literature. The origin of the infection is presumed to be a paravaginal hematoma. However, the actual presence of a paravaginal hematoma with subsequent abscess formation and spread through the pelvis into the lower extremity has never been previously demonstrated. Additionally, obturator internus muscle hematoma with or without infection has also not been reported previously in the absence of pudendal or paracervical block anesthesia. Hibbard et al.[2] have described the possible route of spread of a paravaginal hematoma abscess. These routes may include a spread through the lesser sciatic foramen towards the hip joint or upward through the broad ligament through the pelvic sidewall. Failure to recognize this rare complication can be associated with severe morbidity or even death.

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6/1/33042

Case report

F.J., a 27-year-old black woman, gravida 2, para 1, with no significant past medical history was seen in the



Fig. 1. A CT scan of pelvis 1 week postpartum shows hematoma *(H)* of right obturator internus muscle deviating bladder *(B)* left.

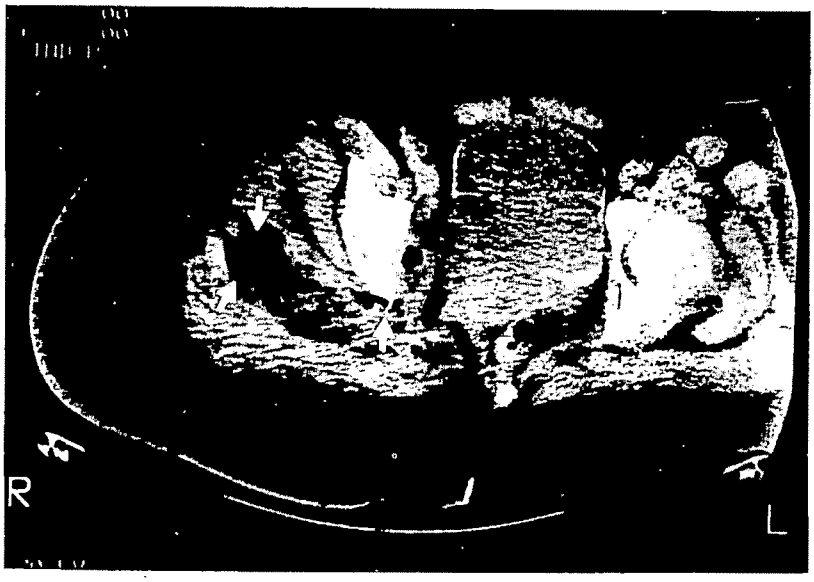


Fig. 2. A CT scan shows gas formation *(arrows)* in obturator internus muscle disseminating into subgluteal region.

labor and delivery suite at 41 weeks' gestation in spontaneous labor after an uneventful prenatal course. She was fully dilated with the vertex at +1 station on admission. She had an uncomplicated normal delivery of a 9-pound female infant after a second stage lasting 1 hour, 5 minutes. No episiotomy was required nor did she receive perineal or pudendal block anesthesia. She had no vaginal or perineal lacerations noted at delivery. The patient was discharged home in satisfactory condition on the third postpartum day.

The patient returned 1 week later complaining of shooting pains in the right buttock radiating down to the back of her thigh. The pelvic examination revealed tenderness along the right ischial spine area with fullness. A computed tomographic (CT) scan of the pelvis was done, which revealed a hematoma of the right obturator internus muscle extending towards the ischial spine (Fig. 1). Admission was recommended for evacuation of the hematoma but was refused by the patient. She returned 1 week later with severe pain in the right leg and inability to walk. Physical examination revealed a very ill patient in excruciating pain. Her temperature was 40.2° C. The right leg was swollen and tender with bullae present around the knee joint and lateral aspect of the thigh. A second CT scan was done, which showed a gas collection extending from the right obturator internus muscle into the subgluteal region (Fig. 2). The scout image from the second CT scan examination demonstrated a large amount of soft tissue gas in the subgluteal region extending into the thigh down to the upper calf (Fig. 3).

The patient was started on ampicillin, gentamicin, and clindamycin and was taken to the operating room where a fasciotomy of the right buttock, thigh, and leg was performed. Necrotic fascia and purulent material were noted to the level of the midcalf. After adequate drainage and debridement, the wound was left open.

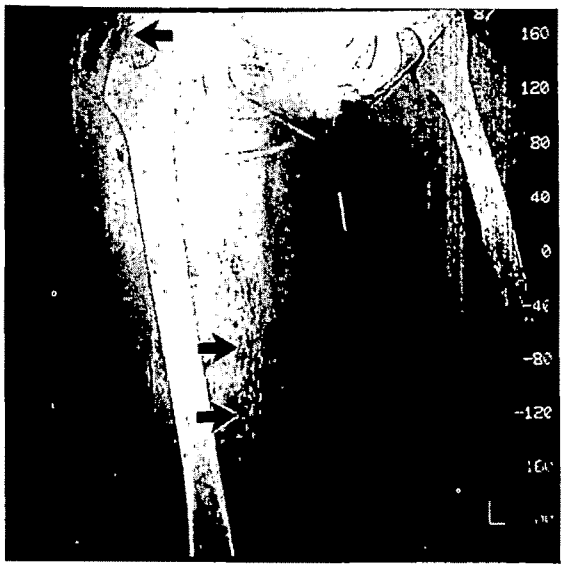


Fig. 3. Scout film for CT scan done 2 weeks postpartum shows gas formation around greater trochanter and disseminated down the leg *(arrows)*.

Cultures of the pus revealed *Escherichia coli* and enterococcus.

After multiple surgical debridements, antibiotic therapy, whirlpool treatment, and extensive physical therapy, the patient was discharged home on the sixty-first hospital day. At the time of discharge, she was fully ambulatory except for some residual discomfort and a slight limp.

Comment

Infection or necrotizing fascitis of the lower extremity after delivery is a rare occurrence. The origin of

this infection is presumed to be a paravaginal hematoma. This complication involving hematoma formation, subsequent infection, and spread to the lower extremity has only been reported with paracervical or pudendal block anesthesia. The present communication describes the first documented case of paravaginal hematoma with subsequent infection and extensions into the lower extremity after an uncomplicated delivery.

The development of a paravaginal hematoma after spontaneous delivery can conceivably occur from rupture or laceration of the pudendal vessel as it courses through the obturator internus fascia. These patients usually remain asymptomatic for a varying period. The initial symptoms are often confused with an orthopedic problem resulting from pregnancy or delivery.

In any paravaginal hematoma, infection can develop by penetrance of vaginal bacteria through small lacerations. Once the hematoma is infected, an abscess may develop. This abscess can extend along the fascia through the lesser sciatic foramen and then along the course of the sciatic nerve down the leg. Alternatively, the infection can spread along the base of the broad ligament to the pelvic sidewall and then through the greater sciatic foramen along the course of the sciatic nerve. An early infection may respond to antibiotics, but, once the destructive abscess has developed and spread has occurred, incision and drainage with debridement of the necrotic tissue along with antibiotic therapy becomes necessary. This case presents a very rare complication of normal vaginal delivery. A postpartum patient with sciatic nerve–type pain should raise the suspicion of a paravaginal hematoma. Prompt investigation with a CT scan and immediate treatment will prevent complication as described herein.

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Interrelationship and clinical significance of increased resistance in the uterine arteries in patients with hypertension or preeclampsia or both

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OBJECTIVES: This study was designed to evaluate the clinical significance of the Doppler flow velocity waveform analysis of the two uterine arteries on an individual basis and in combination expressed as the mean uterine artery.

STUDY DESIGN: We evaluated uterine artery resistance by means of continuous wave Doppler ultrasonography in 123 pregnant women with chronic hypertension, preeclampsia, or both. The placental location was determined by real-time ultrasonography. Clinical outcomes were compared according to uterine artery abnormalities. The Doppler flow studies were not used in patient management.

RESULTS: In patients with unilateral placentas ($n = 67$) the placental uterine artery was found to be a better predictor of poor pregnancy outcome than the nonplacental artery and the mean of the two arteries. There was a strong degree of correlation between abnormal nonplacental uterine artery and abnormal mean of uterine artery ($r = 0.75$, $p < 0.001$), and there was a moderate degree of correlation between abnormal placental uterine artery and abnormal mean uterine artery ($r = 0.46$, $p < 0.001$). Uterine artery discordance (left − right uterine artery systolic/diastolic ratio) was mostly the result of an abnormal nonplacental uterine artery ($r = 0.74$, $p < 0.0001$) and not the result of an abnormal mean uterine artery ($r = 0.44$, $p < 0.003$); the degree of discordance dic not relate to pregnancy outcome. Unilateral placental location was associated with longer stays in neonatal intensive care units and more perinatal deaths.

CONCLUSION: Because of the differences between the two uterine arteries, we conclude that for proper interpretation of uterine artery flow velocity waveforms, the placental location should be known and each vessel analyzed individually. (Am J Obstet Gynecol 1992;166:601-6.)

Key words: Uterine arteries, hypertension, preeclampsia, Doppler ultrasonography

Intrauterine growth retardation and preeclampsia are both conditions strongly related to proper placental development and function. It has been shown that poor trophoblastic conversion of the spiral arterioles leads to development of intrauterine growth retardation, preeclampsia, or both.[1] Poor trophoblastic conversion results in a decreased uteroplacental vascular capacitance and increased uteroplacental artery resistance. This in turn can lead to decreased uteroplacental bed perfusion, because perfusion is inversely proportional to vascular resistance.

Doppler flow velocity waveform analysis in the uterine arteries performed by continuous wave Doppler ultrasonography is considered a clinically useful method for evaluation of high-risk pregnancies, especially in pregnancies complicated by impaired fetal growth and preeclampsia.[2-7] Some investigators have evaluated only the placental uterine artery and others both uterine arteries collectively. Schulman et al.[8] have shown that both uterine arteries do not respond to pregnancy with the same degree of compliance and that the difference in the resistance (change in systolic/diastolic ratio) between the two uterine arteries correlated well with poor pregnancy outcome. We have shown that placental location can influence uterine artery resistance.[9] Placental laterality is associated with elevated uterine artery resistance and the development of preeclampsia and impaired fetal growth.[10]

The present study was designed to evaluate the relationship between pregnancy outcome and increased resistance in each uterine artery individually and the interrelationship of the two uterine arteries in relation to clinical outcome in patients with hypertension and preeclampsia or both.

Material and methods

A total of 160 patients were enrolled in the study. Seventeen were excluded because of incomplete pregnancy outcome information and 20 because the interval from the last study to delivery was >10 days. The study subjects were recruited from a patient population referred to the antenatal testing unit of Forsyth Memorial Hospital for fetal evaluation because of preeclampsia or chronic hypertension. All patients were examined

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Table I. Clinical and Doppler characteristics in seven cases with perinatal mortality

Case No.	*Diagnosis*	*Gestational age at delivery (wk)*	*Birth weight (gm)*	*Uterine artery flow velocity waveform*	*Umbilical artery flow velocity waveform*	*Location of placenta*
1	Chronic hypertension	26	480	Abnormal	Normal	Central
2	Superimposed preeclampsia	28	520	Abnormal	Abnormal	Lateral
3	Preeclampsia	26	560	Abnormal	Abnormal	Central
4	Chronic hypertension	35	588	Abnormal	Abnormal	Lateral
5	Preeclampsia	28	665	Abnormal	Abnormal	Lateral
6	Chronic hypertension	39	3544	Normal	Normal	Lateral
7	Preeclampsia	32	1180	Abnormal	Abnormal	Lateral

*Electronic fetal monitoring.

after 24 weeks' gestation and no more than 10 days before delivery. Although the majority of the patients underwent more than one evaluation, only the last examination was used in the analysis, because our goal was to examine the relationship of the uterine artery condition to pregnancy outcome as close to delivery as possible. The majority of the patients were inpatients (105) and the rest were outpatients. The physicians involved in the management of the patients were unaware of the Doppler data, and all management decisions were based solely on standard obstetric care practices. The study was approved by the institution's Clinical Research Practices Committee; all patients gave written informed consent.

Diagnosis and classification of the hypertensive disorders were according to criteria established by the American College of Obstetricians and Gynecologists.[11] Patients were considered to have pathologic proteinuria if the total protein level in a 24-hour urine specimen was ≥500 mg or if a random urine specimen obtained by bladder catheterization had a dipstick value of ≥2+. Growth charts appropriate for our population were used to classify the infants as small for gestational age or appropriate for gestational age.[12]

The placental location was determined by real-time ultrasonography.[9] The flow velocity waveforms were obtained with the mother lying comfortably in a slight lateral tilt according to a previously described method.[9] A 4 MHz continuous-wave Doppler device equipped with a real-time spectrum analyzer was used to obtain the uterine artery flow velocity waveforms. The systolic/diastolic ratio was used as an index of resistance in the vasculature distal to the point of insonation. The average of two measurements on each side was classified as right and left uterine artery flow velocity waveform, respectively. When necessary for comparisons, the mean of the right and left uterine arteries was classified as the mean uterine artery flow velocity waveform. In patients with unilateral placenta the uterine artery ipsilateral to the placenta was classified as placental uterine artery and the one contralateral to the placenta as nonplacental uterine artery.

For comparison with previous reports the entire sample ($n = 123$) was analyzed according to the mean uterine artery flow velocity waveform without consideration of the placental location. Whenever we refer to placental and nonplacental uterine artery we consider only the patients with unilateral placentas ($n = 67$), excluding the patients with central placentas.

The flow velocity waveforms were classified as abnormal if there was dicrotic notching in early diastole or if the respective value of the systolic/diastolic ratio exceeded the 95th percentile of our normal values or both. In patients with unilateral placentas the established value is 2.3 for the placental and 3.7 for the nonplacental uterine artery. In patients with central placentas this value is 2.8 and represents the mean of the two uterine arteries.[13] The difference between the right and left uterine artery systolic/diastolic ratio was termed uterine artery discordance.

Statistical analysis was performed by χ^2 test for comparison of frequencies of various pregnancy outcomes. The relationship of placental, nonplacental, and mean uterine arteries was explored with Pearson's correlation coefficient. Comparisons of clinical and Doppler parameters between groups were performed by Student t test for independent samples with unequal variances.

Results

Data from 123 patients were analyzed. Of those 123 patients, 67 had unilateral placentas and 56 had central placentas. There were no differences between the two groups in regard to gravidity, parity, degree of hypertension, degree of proteinuria, gestational age at delivery, and birth weight. However, the uterine artery discordance and the length of stay in the neonatal intensive care unit were different ($p < 0.05$ and $p = 0.05$, respectively). There were seven perinatal deaths in this group of 123 patients with five (72%) occurring in patients with unilateral placentas (Table I). Comparison of patients according to abnormality in the three uterine vessel subgroups (normal vs abnormal mean uterine artery flow velocity waveform, normal vs abnormal placental uterine artery, and normal vs abnormal nonpla-

*Antepartum test result**	*Maternal mean arterial pressure (mm Hg)*	*Apgar score*	*Indication for delivery*
Abnormal	84	1	Fetal distress
Abnormal	110	8	Fetal distress
Not done	123	0	Intrauterine fetal death
Normal	94	0	Intrauterine fetal death
Abnormal	120	9	Fetal distress
Normal	97	0	Intrauterine fetal death
Abnormal	110	8	Fetal distress

cental uterine artery revealed no differences in the degree of proteinuria or hypertension.

Tables II through IV demonstrate comparisons of clinical outcomes between normal and abnormal uterine arteries. It is evident that an abnormal placental uterine artery is the best predictor of poor outcome, the abnormal mean uterine artery is second best, and the nonplacental uterine artery was the poorest predictor of clinical outcome. Eighty patients underwent delivery by cesarean section. The majority of them (82%) did so for fetal or maternal indications directly related to the hypertensive disorders and only 18% for obstetric indications not related to the patients' hypertensive disorder. This was the case in all groups of patients. Table V lists the indications for cesarean deliveries. All patients who delivered before 37 completed weeks ($n = 62$) did so because of fetal or maternal indications related to the hypertensive disorder.

The presence of notching in the placental uterine artery regardless of systolic/diastolic value is associated with lower birth weight in neonates of similar gestational age at delivery ($p < 0.02$). In comparing the degree of discordance between right and left uterine arteries in patients with notching and those without, we found that placental uterine artery notching does not affect the degree of discordance between the two uterine arteries in the two groups ($p = 0.7$). However, the presence of notching in the nonplacental uterine artery is associated with a greater discordance between right and left uterine arteries ($p < 0.0001$). Regression analysis of the degree of discordance (change in systolic/diastolic ratio) on the systolic/diastolic ratio of the placental and nonplacental uterine artery and mean uterine artery flow velocity waveform from all patients revealed no correlation between uterine artery discordance and placental uterine artery resistance ($r = -0.09$, $p = 0.5$). To the contrary, there exists a significant correlation between uterine artery discordance and nonplacental uterine artery resistance ($r = 0.74$, $p < 0.0001$). The mean resistance correlates also with the uterine artery discordance but to a lesser degree ($r = 0.44$, $p < 0.003$). Comparisons of clinical characteristics according to the degree of discordance (change in systolic/diastolic = 1, 1.5, and 2) failed to disclose any statistically significant differences, except that the values of the mean uterine artery flow velocity waveform and nonplacental uterine artery were significantly higher in patients with higher discordance. This was true for the entire group and in the group with unilateral placenta. Uterine artery discordance became clinically discriminatory for poor pregnancy outcome only in association with an abnormal mean uterine artery flow velocity waveform.

Seventy-five percent of the patients with abnormal placental uterine artery also have abnormal mean uterine artery flow velocity waveform ($p < 0.004$), and 81% of those with abnormal nonplacental uterine artery also have abnormal mean uterine artery flow velocity waveform ($p < 0.0001$). Thirty percent of the patients with normal placental uterine artery have abnormal mean uterine artery flow velocity waveform ($p < 0.004$), in contrast to only 4% of the patients with normal nonplacental uterine artery who have abnormal mean uterine artery flow velocity waveform ($p < 0.0001$). Abnormal mean uterine artery flow velocity waveform correlates better with abnormal nonplacental uterine artery than with an abnormal placental uterine artery ($r = 0.75$, $p < 0.0001$ and $r = 0.46$, $p < 0.001$, respectively).

Comment

This is the first report to evaluate the clinical significance of abnormal uterine artery resistance for each uterine artery separately in relation to placental location. Previous reports have examined the clinical significance of the subplacental arcuate artery,[6] either one of the two ascending uterine artery branches,[14] or the average of the two uterine arteries.[5] As expected, the results were variable. In part the differences are due to different methods used by various investigators and, to a significant extent, to the differences between the two uterine arteries.

Trudinger et al.[6] limited signal sampling only in the subplacental bed where one can only identify signals from radial and arcuate arteries. Either one of these vessels individually is responsible for only a limited portion of placental bed perfusion and as such it may very well be normal in the presence of significant placental pathology in parts of the placental bed perfused by other arcuate and radial arteries. An abnormal subplacental flow velocity waveform, however, is more likely to be associated with placental pathology because the bulk of blood volume that flows through the subplacental radial and arcuate arteries is directed toward the placental spiral arteries.

Campbell et al.[14] evaluated both ascending uterine arteries with pulsed-wave Doppler and used the most abnormal vessel (highest resistance). From their description and technique it is very likely that some times

Table II. Comparison of clinical outcomes in patients with normal versus abnormal mean uterine artery resistance

Clinical outcomes	*Mean uterine artery status*				*Significance*
	Abnormal (n = 79, 100%)		*Normal (n = 44, 100%)*		
Small for gestational age	28	(35%)	8	(18%)	NS
5 min Apgar score <7	7	(9%)	1	(2%)	NS
Preterm delivery	52	(66%)	10	(23%)	$p < 0.001$
Cesarean delivery	58	(73%)	22	(50%)	$p < 0.02$
Perinatal deaths	6	(7.6%)	1	(2%)	NS
Newborn intensive care unit stay	24	(30%)	1	(2%)	$p < 0.001$
Patients with proteinuria	35	(44%)	17	(39%)	NS

NS, Not significant.

Table III. Comparison of clinical outcomes in patients with normal versus abnormal placental uterine artery resistance

Clinical outcomes	*Placental uterine artery status*				*Significance*
	Abnormal, (n = 40, 100%)		*Normal (n = 27, 100%)*		
Small for gestational age	17	(43%)	3	(11%)	$p < 0.03$
5 min Apgar score <7	4	(10%)	2	(7%)	NS
Preterm delivery	26	(65%)	9	(33%)	$p < 0.03$
Cesarean delivery	33	(83%)	16	(60%)	$p < 0.05$
Perinatal deaths	4	(10%)	1	(3.7%)	NS
Neonatal intensive care unit stay	11	(28%)	4	(15%)	$p < 0.001$
Patients with proteinuria	17	(42%)	17	(62%)	NS

NS, Not significant.

they obtained placental and other times nonplacental uterine artery signals. By this method they achieved good sensitivity in predicting problem pregnancies remote from term (18 to 20 weeks' gestation), although the positive predictive value of an abnormal outcome was low. Steel et al.[7] examined one of the two uterine arteries without prior knowledge of the placental location to screen patients at risk for preeclampsia. Abnormal uterine artery resistance at 24 weeks' gestation identified correctly 63% of the patients who subsequently developed preeclampsia. McParland et al.[15] in a subsequent study, treated patients who had abnormal uterine artery resistance with 75 mg aspirin every day in a random, double-blind fashion. The group of patients who were treated presented with a lower incidence of pregnancy-induced hypertension, proteinuric preeclampsia, and intrauterine growth retardation.

Our data suggest that an abnormal placental uterine artery is the best predictor of poor pregnancy outcome, whereas the average of both uterine arteries is the next best and the nonplacental is the least. Several previous reports have clearly demonstrated that the placental uterine artery has a significantly lower resistance than the nonplacental uterine artery.[8, 16, 17] Therefore, when the placental uterine artery is abnormal, there must be a significant degree of placental abnormality. In addition it should be kept in mind that the placental uterine artery reflects pathologic conditions of the majority of the placental bed vasculature. On the other hand, the nonplacental uterine artery is perfusing primarily nonplacental myometrial vessels to the extent that the placenta is unilaterally located.

It seems that in a group of patients with hypertensive disorders and similar risks for poor outcome the presence of a unilateral placenta increases the risk for perinatal death. Seventy-two percent of perinatal deaths occurred in the group with unilateral placentas and only 28% in the group with centrally located placentas. A unilateral placenta was also found to be associated with longer stay in the newborn intensive care unit and increased degree of discordance between the two uterine arteries. Schulman et al.[8] reported significantly increased risk for poor pregnancy outcome with increasing uterine artery discordance. They also found a significant degree of correlation between mean uterine artery flow velocity waveform systolic/diastolic ratio and change in systolic/diastolic ratio (left − right uterine artery systolic/diastolic ratio). Our findings are partially in agreement with regard to poor pregnancy outcome. However, our data demonstrate a moderate degree of correlation between mean uterine artery flow velocity waveform systolic/diastolic ratio and change in

Table IV. Comparison of clinical outcomes in patients with normal versus abnormal nonplacental uterine artery resistance

Clinical outcomes	*Nonplacental uterine artery status*				*Significance*
	Abnormal (n = 47, 100%)		*Normal (n = 20, 100%)*		
Small for gestational age	17	(36%)	3	(15%)	NS
5 min Apgar score	4	(9%)	2	(10%)	NS
Preterm delivery	30	(64%)	5	(25%)	$p < 0.04$
Cesarean delivery	37	(79%)	12	(60%)	NS
Perinatal deaths	4	(8.5%)	1	(5%)	NS
Neonatal intensive care unit stay	15	(32%)	2	(10%)	NS
Patients with proteinuria	22	(46%)	11	(55%)	NS

NS, Not significant.

Table V. Indications for cesarean section and number of patients in each category

Indication	*No. of patients*	*Proportion of total (n = 123) (%)*
Fetal distress	30*	24
Severe preeclampsia	36*	29
Repeat cesarean section	5	4
Diabetes mellitus	4	3
Cephalopelvic disproportion	4	3
Genital herpes	1	1
TOTAL	80	65

*Sixty-two of these 66 patients were delivered before 37 completed weeks' gestation.

systolic/diastolic ratio. In contrast, there exists a significant degree of correlation between the nonplacental uterine artery systolic/diastolic ratio and the change in systolic/diastolic ratio. The difference may be due to different methodology. Schulman et al.[8] considered as abnormal a systolic/diastolic ratio of ≥2.8 for both placental and nonplacental uterine arteries. We do not concur with this method, since the 95th percentile of the systolic/diastolic ratio for the placental uterine artery is 2.3 and for the nonplacental uterine artery is 3.7 in our normal population.[12] Our data suggest that the degree of abnormality of the mean uterine artery flow velocity waveform is strongly influenced by the nonplacental uterine artery in patients with unilateral placenta and thus may lose some of its sensitivity because the nonplacental uterine artery may be abnormal in the absence of significant placental pathology.

We conclude that in patients with hypertension, placental uterine artery resistance is a better predictor of poor outcome than the mean and the nonplacental uterine artery. In the same patients the presence of a unilateral placenta is a significant risk factor associated with increased perinatal morbidity and mortality. To extract the best information from uterine artery flow velocity waveform analysis, it is important that the placental location is known and each uterine artery is evaluated individually. For this purpose appropriate reference values that account for placental location should be used. Increasing use of ultrasonographic equipment with Doppler capabilities will render this more feasible and thus improve the level of information about the uteroplacental vasculature in patients with hypertensive disorders.

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Direct inhibition of proteases and cervical plasminogen activator by antibiotics

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OBJECTIVES: Preterm premature rupture of the fetal membranes and premature delivery are sometimes linked to genital tract infection and activation of proteolytic enzymes that degrade the extracellular matrix. The possible beneficial effect of antibiotics in prevention of preterm premature rupture of fetal membranes and retardation of the onset of labor in some patients with clinical or subclinical infection was explained via their antibacterial efficacy. The aim of this study was to determine the effect of antibiotics on proteolytic enzymes as a possible explanation for the ability of antibiotics to retard preterm labor.
STUDY DESIGN: The direct effect of four antibiotics on the proteolytic activities of purified collagenase, elastase, plasmin, trypsin, and chymotrypsin and on streptokinase and human cervical plasminogen activator was measured.
RESULTS: The macrolide antibiotic erythromycin and the beta-lactam antibiotics penicillin G, cloxacillin, and ampicillin exerted, in most of the tested combinations with the different proteases, inhibitory effects on the proteolytic activities.
CONCLUSION: The present finding that antibiotics directly inhibit proteases may offer an explanation for the beneficial response to antibiotic therapy in some cases of idiopathic preterm labor even in absence of pathogenic bacterial infection. (Am J Obstet Gynecol 1992;166:606-12.)

Key words: Preterm premature rupture of the membranes, preterm delivery, plasminogen activator, protease, antibiotics

Preterm premature rupture of the fetal membranes and idiopathic preterm labor are two important factors that provoke fetal prematurity. Although the pathogenesis of preterm premature rupture of membranes is still incompletely understood, some studies suggest that bacterial infections of the genital tract and the associated inflammatory responses are factors that either directly or by predisposition contribute to the initiation of preterm premature rupture of membranes and premature delivery.[1,2] The microorganisms and the neutrophils and macrophages that localize at sites of inflammation produce various hydrolytic enzymes, including proteases, collagenases, elastases, and phospholipases.[1-5] These enzymes can degrade chorionic membrane components and thereby reduce the membranal strength.[5] Bacterial genital infection with *Staph-*

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ylococcus aureus, group B streptococci, bacterial vaginosis, and cervical infection with *Chlamydia trachomatis* during pregnancy are known risk factors for preterm premature rupture of membranes.[3, 6, 7] A possible association between infections of the lower and upper genital tract of women, inflammation, and preterm birth is also supported by other studies.[8, 9]

Normal parturition is preceded by structural changes of the cervical matrix leading to softening, dilatation, effacement, and distensibility. These changes result from enhanced hydrolytic cleavage of matrix proteins by proteolytic enzymes such as elastase and collagenase.[10] Elastase is an endopeptidase unique in its ability to cleave the elastic fibrous protein of connective tissue,[11] whereas collagenase has the capacity to cleave native collagen.[12] Increased activity of collagenase occurs concomitantly with cervical dilatation and ripening in human parturition.[10, 13] Both elastase and collagenase are produced from inactive zymogens by proteolytic cleavages that remove a small peptide from the inactive zymogens, thereby creating the active configuration of the enzymes. The endopeptidase plasmin is capable of this activation. Plasmin itself is produced from inactive plasminogen through a specific cleavage by plasminogen activator(s), often present in inflamed tissues.[14] The plasminogen activator(s) and the other proteases may originate from bacteria or leukocytic infiltration that occurs before parturition.[4, 15]

Although entry of vaginal microorganisms into the cervix and lower uterine tissues has been suggested as inducing preterm labor, treatment with antibiotics such as erythromycin was reported to prolong pregnancy in women with cervical effacement and dilatation.[9] Because even the normal cervicovaginal flora produces proteases capable of hydrolyzing the extracellular matrix, a common strategy for prevention of preterm premature rupture of membranes is treatment with antibiotics, even in absence of overt infection. Recently, McGregor et al.[16] reported that bacteria-induced weakening of the fetal membrane can be prevented by levels of antitiotics below those needed to stop bacterial growth, probably reflecting an antibiotic-mediated inhibition of bacterial protease production or release.

Because direct inhibitory effects of certain antibiotics on some proteolytic enzymes were observed earlier,[17-19] it is possible that the proteases that damage the membranes can be directly inhibited by particular antibiotics. Therefore we have determined the effect of several antibiotics on the activities of some purified proteases and on the plasminogen activator activity derived from human cervical tissue.

Material and methods

Materials. Plasminogen was obtained from outdated human plasma by the method described by Deutsch and Mertz[20] with agarose-L-lysine (Bio Yeda, Rehovot). Carbon 14–labeled globin was prepared by formation of carbaminohemoglobin with ^{14}C-labeled potassium cyanate, followed by separation of the globin from heme.[21] Hemoglobin (type III), trypsin from bovine pancreas, chymotrypsin from bovine pancreas, elastase (type IV) from porcine pancreas, and collagenase (type IV) from *Clostridium histolyticum* were purchased from Sigma Chemical Co., St. Louis. Labeled potassium cyanate was obtained from New England Nuclear, Boston; human plasmin from Kabi Diagnostica, Stockholm; streptokinase (Streptase) derived from β-hemolytic streptococci of Lancefield group C from Behringwerke Ag, Marburg; ampicillin (sodium salt) and penicillin G (benzylpenicillin sodium) from Teva, Israel; cloxacillin (sodium) from Bristol Italiana (Sud), Italy; and erythromycin lactobionate (Erythrocin; erythromycin mono (4-O-β-D-galactopyranosyl-D-gluconate) from Abbott Laboratories, North Chicago.

Cervix-conditioned media. Cervical tissue samples were obtained from uteri of women undergoing hysterectomy for various gynecologic indications. The tissue was transferred to the laboratory in ice-cold saline solution and washed with Tris buffer (0.2 mol/L, adjusted to pH 8.0 with hydrochloric acid). Conditioning of the buffer was performed by incubating sections of 1 gm of tissue with 10 ml of the Tris buffer for 2 hours at 37° C. The relative contribution of tissue-type plasminogen activator and non-tissue-type plasminogen activator activities to the total plasminogen activator activity emerging in the conditioned buffer was assessed by inhibition experiments with specific antihuman melanoma tissue-type plasminogen activator antibodies, kindly provided by Dr. Yoav Sharoni, Ben-Gurion University, Beer Sheva.

Globinolytic assay of plasminogen activator activity. Plasminogen activator activity was measured in aliquots of 0.4 ml of the conditioned buffer. Plasminogen, 10 μl of a stock solution of 10 mg/ml, was preincubated for 30 minutes at 37° C with the conditioned buffer. Subsequently, 25 μl of ^{14}C-globin, 336,000 counts/min/mg, 12.5 mg/ml, was added and incubation was continued for 30 minutes. Activity of streptokinase was similarly measured by incubation of purified streptokinase, 10 IU, in 0.4 ml of Tris hydrochloride buffer, 0.2 mol/L, pH 8.0, with globin and plasminogen at the above-stated concentrations for 30 minutes. The reactions were terminated by addition of 250 μl of 50% (wt/wt) ice-cold trichloroacetic acid, and the acid-soluble radioactivity was measured. With this assay the ability of different antibiotics to inhibit plasminogen activator activity of cervix-conditioned medium or purified streptokinase was assayed at increasing levels of antibiotics, ranging from about 0.004 to about 10 mg/ml (10^{-5} to 10^{-2} mol/L).

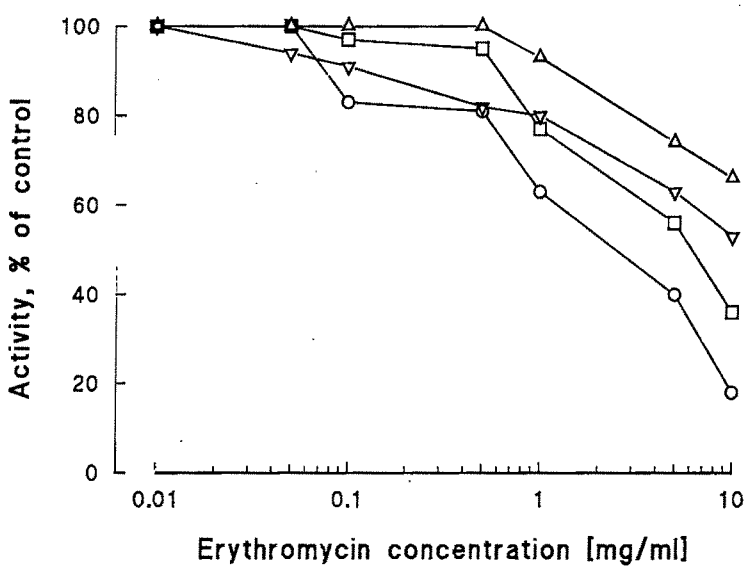


Fig. 1. Effect of increasing concentrations of erythromycin on activity of proteases. Globinolytic activity of 2.5 milli–casein units plasmin (△), 6.7 mU elastase (□), 28.5 U collagenase (○), or 10 IU streptokinase (▽) was determined during 30 minutes of incubation at 37° C. Activity in absence of erythromycin was taken as 100%.

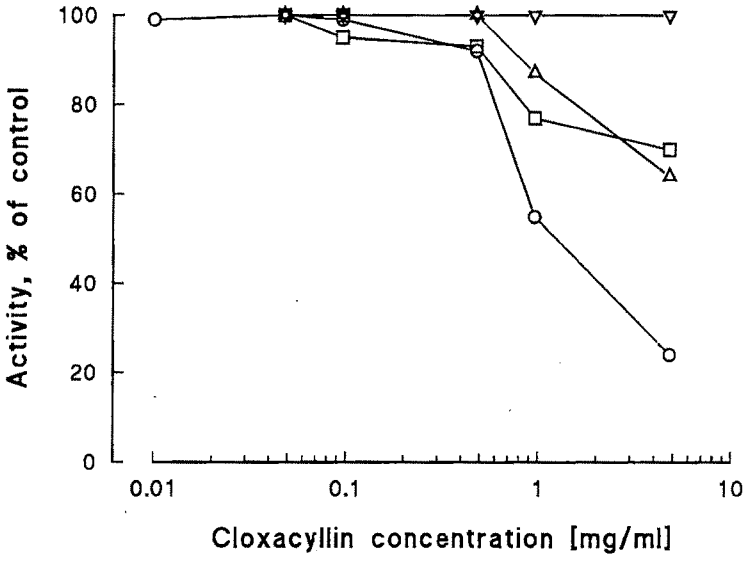


Fig. 2. Effect of increasing concentrations of cloxacillin on activity of proteases. Globinolytic activity of 2.5 milli–casein units plasmin (△), 6.7 mU elastase (□), 28.5 U collagenase (○), or 10 IU of streptokinase (▽) was determined during 30 minutes of incubation at 37° C. Activity in absence of cloxacillin was taken as 100%.

Inhibition of purified proteases by antibiotics. The inhibitory activities of penicillin G, cloxacillin, ampicillin, and erythromycin on the purified enzymes plasmin (2.5 milli–casein units), trypsin (4 IU), chymotrypsin (6.5 mU), collagenase (28.5 U), and elastase (6.7 mU) were determined. Aliquots of 0.4 ml of Tris hydrochloride buffer, 0.2 mol/L, pH 8.0, with or without antibiotic at increasing concentration from 0.005 or 0.1 mg/ml up to 10 mg/ml (i.e., from 10^{-5} up to 10^{-2} mol/L) were incubated with 25 μl of ^{14}C-labeled globin, 16 mg/ml. Incubation was at 37° C for 30 minutes. The reaction was terminated by addition of 250 μl of 50% (wt/wt) ice-cold trichloroacetic acid. The acid-soluble radioactivity was measured.

Kinetic parameters. The kinetic parameters of the different enzymes were established by the globinolytic assay. Collagenase activity was tested by incubating 28.5 U of collagenase in the presence or absence of antibiotic

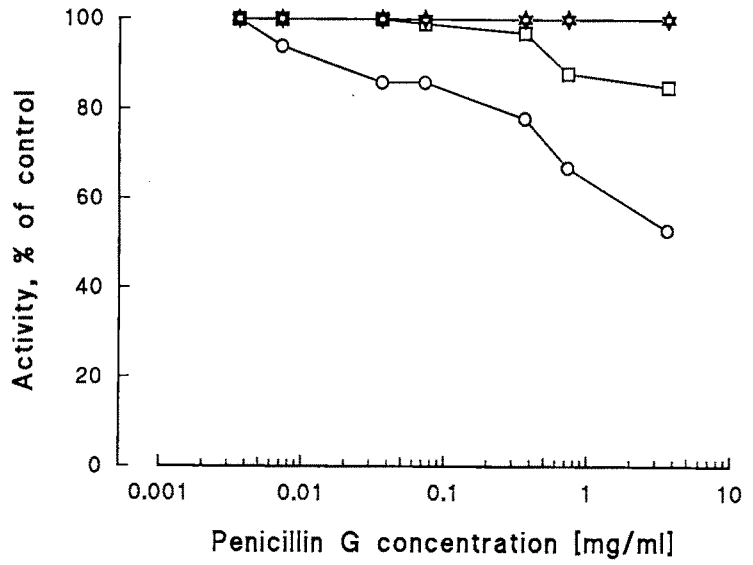


Fig. 3. Effect of increasing concentration of penicillin G on activity of proteases. Globinolytic activity of 2.5 milli–casein units plasmin (△), 6.7 mU elastase (□), 28.5 U collagenase (○), or 10 IU streptokinase (▽) was determined during 30 minutes of incubation at 37° C. Activity in absence of penicillin G was taken as 100%.

in a concentration of 1×10^{-2} mol/L. The enzyme was incubated with different concentrations of the substrate ^{14}C-globin, ranging from 3×10^{-6} to 30×10^{-6} mol/L, in a total volume of 0.4 ml. Cervical plasminogen activator in the conditioned buffer was tested by the globinolytic assay. Conditoned buffer was incubated in the absence or presence of 0.5 mg/ml erythromycin or 0.1 mg/ml cloxacillin (5×10^{-4} mol/L) or 3.6 mg/ml penicillin G (1×10^{-2} mol/L). To determine the type of inhibition, cervical plasminogen activator was incubated with increasing concentration of plasminogen, from 0.75×10^{-7} to 7.6×10^{-7} mol/L.

All experiments were performed in duplicate, and each experiment was run at least three times. Results are taken from representative experiments and are mean values of the duplicate. Variation between reruns of the same experiment never exceeded 7%.

Results

The response of several purified proteolytic enzymes and the response of human cervical plasminogen activator to antibiotic drugs was established in a series of experiments. In these experiments the tested purified enzymes were collagenase, elastase, plasmin, trypsin, and chymotrypsin. The antibiotics used were the macrolide antibiotic erythromycin and the three β-lactams penicillin G, cloxacillin, and ampicillin. All the compounds were tested in a wide concentration range, which included the levels commonly obtained in the plasma during treatment. Fig. 1 shows the effect of increasing concentrations of erythromycin on the activities of the purified proteases. Erythromycin is noted to inhibit the activity of collagenase, elastase, streptokinase, and plasmin. The greatest inhibitory effect occurs on collagenase, followed by a smaller inhibitory effect on elastase and streptokinase, and the lowest inhibition is noted on plasmin activity. The response of the same enzymes to the β-lactam antibiotics cloxacillin and penicillin G is depicted in Figs. 2 and 3, respectively. Again, collagenase and elastase responded with the largest extent of inhibition. Streptokinase activity is refractory to cloxacillin (Fig. 2) and to penicillin (Fig. 3), whereas plasmin activity is inhibited by cloxacillin (Fig. 2) but not by penicillin G (Fig. 3). Other experiments demonstrated that the β-lactam antibiotic ampicillin at concentration >0.37 mg/ml (1×10^{-3} mol/L) inhibits collagenase by 30% and elastase by 20% but has no effect on plasmin or streptokinase. Two additional enzymes, the serine proteases trypsin and chymotrypsin, are also inhibited by erythromycin and cloxacillin; 50% inhibition of trypsin and 65% inhibition of chymotrypsin are obtained with 5 mg/ml (5×10^{-3} mol/L) of erythromycin. A similar concentration of cloxacillin inhibits both enzymes by 20%. In contrast, penicillin G and ampicillin do not inhibit trypsin and cause only a slight inhibition (10%) of chymotrypsin activity, and only at the high concentration of 3.6 mg/ml (1×10^{-2} mol/L).

The results described indicated that among the four tested proteases collagenase is the most sensitive to inhibition by antibiotics. The kinetic parameters of collagenase in absence and in presence of the antibiotics are summarized in Table I. Erythromycin, penicillin G, and cloxacillin are seen to be noncompetitive inhibitors.

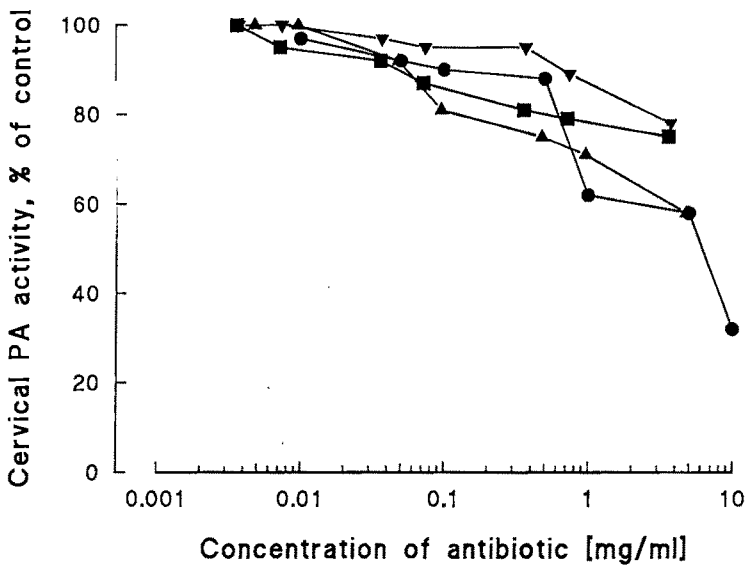


Fig. 4. Effect of different antibiotics on globinolytic activity of human cervical plasminogen activator *(PA)*. Increasing amounts of ampicillin (▼), penicillin G (■), cloxacillin (▲), or erythromycin (●) were incubated in 0.4 ml aliquots of medium conditioned with human cervix. The plasminogen activator activity of cervix-conditioned buffer was assayed by addition of 10 μl plasminogen for 30 minutes at 37° C. Subsequently, 25 μl of ^{14}C-globin was added and incubation was continued for additional 30 minutes.

Table I. Kinetic parameters for collagenase activity in presence of different antibiotics

Antibiotic	*Maximal velocity of reaction* ($pmol \cdot min^{-1} \cdot U^{-1}$)	*Michaelis-Menten constant* ($\mu mol \cdot L^{-1}$)
None	4.0	16.8
Erythromycin (10.0 mg/ml)	2.4	18.6
Penicillin G (3.6 mg/ml)	3.1	20.7
Cloxacillin (4.8 mg/ml)	2.3	20.7
Ampicillin (3.7 mg/ml)	2.7	11.4

Collagenase, 28.5 U, was incubated at 37° C for 30 minutes in absence or presence of 1×10^{-2} mol/L of different antibiotics. Total volume was 0.4 ml. Collagenase activity was assayed with ^{14}C-globin as substrate in a range of concentrations between 3×10^{-6} and 3×10^{-5} mol/L. Number in parentheses are concentrations of the tested antibiotics.

The v (initial reaction velocity) versus v/[S] plots for these three compounds intersect to the left of the v axis, suggesting that they do not affect the binding of the substrate to collagenase. In contrast, ampicillin is an uncompetitive inhibitor of collagenase, and increasing concentrations of ampicillin yield a set of parallel curves in reciprocal v versus [S] plots (not shown).

To test the effect of antibiotics on cervical plasminogen activator activity, the enzyme was tested in buffer conditioned with freshly obtained human cervical tissue. The plasminogen activator activity recovered from the cervix-conditioned buffer is composed of 80% tissue-type and 20% non-tissue-type plasminogen activator activity. This conclusion is based on measurements of the degree of inhibition of cervical plasminogen activator activity by antihuman tissue-type plasminogen activator antibodies. The effect of four antibiotics on the total plasminogen activator activity in the buffer conditioned with human cervix is shown in Fig. 4. The activity is inhibited by all tested compounds. The largest inhibitory effect is noted with erythromycin, whereas the lowest degree of inhibition is exerted by ampicillin.

Kinetic analysis of the effect of antibiotics on human cervical plasminogen activator activity showed that penicillin G, cloxacillin, and erythromycin do not affect the maximal velocity of the reaction but increase the Michaelis-Menten constant, suggesting a mechanism of competitive inhibition. These kinetic assays were performed by the dual-stage, plasminogen-dependent test, with the use of levels of erythromycin and cloxacillin that are below those that inhibit human plasmin, e.g., 0.5 and 0.1 mg/ml (5×10^{-4} mol/L), respectively. Penicillin G, which does not inhibit human plasmin, was tested at 3.6 mg/ml (1×10^{-2} mol/L). Concordantly, the increased Michaelis-Menten constant reflects an effect on plasminogen activation and not on plasmin activity.

Comment

These findings show that the activities of proteases that are considered to play a role in preterm premature

rupture of membranes and cervical ripening[1, 3, 10, 13, 15] are directly inhibited by several antibiotics. Therefore the ability of antibiotics to offer a certain protective effect against preterm premature rupture of membranes and their capacity to protract premature delivery may be partially ascribed to their ability to suppress proteases that mediate these processes. Thus, regardless of the source of the proteases, either from normal bacterial flora of the genital tract or from pathogenic bacterial infections or even from inflammatory granulocytes or monocytes-macrophages that invade the tissue, the different antibiotics can partially suppress hydrolytic activity. It has already been suggested that antibiotics extend the time from treatment to delivery in patients with idiopathic preterm labor and cervical dilatation.[9] McGregor et al.[16] noted that erythromycin at a level that is subinhibitory to bacterial growth effectively prevents bacteria-induced reduction of fetal membrane strength. This effect suggested an antibiotic-mediated inhibition of the production or secretion of bacterial proteases.[16] Likewise, the administration of ampicillin or erythromycin, even in absence of clinical signs of infection, results in delayed delivery.[22] The currently described inhibition of proteases by antibiotics may offer an additional explanation for the ability of certain antibiotics to support the integrity of fetal membranes.

We have noted earlier that urokinase activity is inhibited by penicillin G in a competitive manner, whereas ampicillin had no effect.[17] It was suggested that the hydrophobic side chain rather than the β-lactam moiety of the antibiotic is responsible for this inhibition. Tissue-type plasminogen activator demonstrated a similar inhibition by several penicillins.[18]

These findings imply that the mechanism of inhibition of collagenase by ampicillin differs from the mode of action of the other tested antibiotics. The difference is indicated by the changes produced by the various antibiotics in the kinetic parameters of collagenase, as summarized in Table I. The values are derived from sets of experiments in which the initial reaction velocity, v, is determined at increasing substrate concentrations. The maximal velocity of the reaction is obtained when the substrate concentration is so high that essentially all the enzyme is saturated. The Michaelis-Menten constant is numerically equal to the substrate concentration at which the initial reaction velocity is half-maximal. As seen in Table I, ampicillin decreases these two factors of collagenase to the same extent, suggesting its binding to the collagenase-substrate complex rather than to the free enzyme.[23] The other three antibiotics exhibit noncompetitive inhibition, suggesting their ability to form a collagenase-substrate-antibiotic complex that is catalytically inactive.[23]

Elastase and collagenase are produced and secreted from cells in latent forms that require activation within the extracellular matrix.[12, 24] This activation can be mediated by various proteases, including trypsin and plasmin. However, only plasmin activates latent collagenase without destruction and appears to be involved in the in vivo activation of latent collagenase.[12] Plasmin itself is the product of activation of the inactive proenzyme plasminogen by plasminogen activators or by traces of preformed plasmin. Finally, the plasminogen activators themselves are often produced as inactive proenzymes that depend on activation for activity.

Since the existence of collagenase and elastase in latent forms represents a mechanism to control their extracellular activity, the ability to control their conversion to active enzymes by inhibition of proteolytic activities in the cervix may be of clinical relevance. Thus direct suppression of the protease-dependent activation of matrix-degrading enzymes by antibiotics may reduce the incidence and delay the onset of cervical dilatation, preterm premature rupture of membranes, and preterm labor.

The importance of bacterial infection in the etiology of preterm birth has been recently disputed on the basis that patients undergoing preterm delivery exhibit normal vagina flora and by the failure of adjunctive antibiotic therapy to prevent preterm labor.[25] This recent observation should now be reexamined in view of the direct effect of antibiotics on the activity of proteases that are most likely involved in the etiology of preterm premature rupture of membranes and preterm labor.

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The effect of fetal sepsis on umbilical cord blood gases

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The relationship between fetal sepsis and acid-base status is unknown. We hypothesized that in utero sepsis would result in fetal metabolic acidemia. In a retrospective study during a 38-month period, the acid-base status at birth of neonates with in utero sepsis, documented by positive blood cultures, was reviewed. Compared with term neonates, preterm neonates had a 22-fold increase in the risk of bacteremia at birth. In spite of this increased risk of sepsis, there was no significant alteration in arterial pH in preterm septic neonates when compared with preterm controls. Fetal sepsis at term was accompanied by a statistically significant reduction in arterial pH (7.21 ± 0.07) compared with controls (7.26 ± 0.06, $p < 0.05$). When controlled for other variables, the decrease in arterial pH at term was correlated with an increased duration of labor (7.3 ± 0.7 in controls vs 10.8 ± 0.9 hours in neonates with sepsis, $p < 0.05$). The classic predictors of chorioamnionitis were found to be poor prognostic indicators of fetal bacteremia. Fetal sepsis at term is associated with a deterioration in the fetal acid-base status and a prolongation of labor. (Am J Obstet Gynecol 1992;166:612-7.)

Key words: Fetal sepsis, fetal acid-base balance, umbilical cord blood gases

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Controversy exists regarding fetal infection in utero and its effect on acid-base status. In the adult, septicemia has been associated with progressive metabolic acidemia.[1] This has lead to the inference that in utero sepsis may have a similar deleterious effect on fetal acid-base balance. Fetal in utero infection has been implicated as a cause of birth asphyxia.[2,3] In contrast, in the

presence of premature rupture of the membranes, chorioamnionitis does not alter umbilical cord pH.[4] Additionally, in the presence of chorioamnionitis, immediate delivery affords no maternal[5] or neonatal[6] advantage when appropriate antibiotic therapy is provided. Thus the exact relationship between fetal sepsis and umbilical cord blood gas parameters is unknown. The purpose of this study was to establish the effect of infection acquired in utero on fetal acid-base status at birth. We hypothesized that in utero sepsis would result in a fetal metabolic acidemia.

Material and methods

The neonatal records of all infants with positive blood cultures within the first 24 hours of life who were admitted to the nursery at Hermann Hospital (Houston) from January 1986 through March 1989 were reviewed. Neonates with positive blood cultures within 24 hours of birth were considered to have acquired their infection in utero. Because of concerns with contamination, infants with cultures that revealed nonaureus staphylococcus were included only if two blood cultures were positive. When mothers with premature rupture of the membranes were treated with antepartum antibiotics, their neonates were excluded from analysis. Umbilical cord arterial and venous blood gas parameters were used to determine the fetal acid-base status at birth. Sixty-six neonates met these criteria. In five newborns with sepsis blood gases could not be determined from the umbilical cord, and these values were unavailable for analysis. Only those neonates with an arterial specimen were included in the study. The maternal obstetric records of the 61 neonates who met the study criteria were then analyzed. Controls were obtained by manually reviewing the maternal and neonatal medical record of the next consecutive delivery; they were specifically matched for maternal age and parity, socioeconomic status, gestational age, birth weight, and mode of delivery. Health care delivery service was used as a marker for socioeconomic status. Patients cared for by the University of Texas Women's Clinic, by a private health maintenance organization, or by private physicians were eligible for inclusion in the study. The following maternal criteria were evaluated: chronic medical conditions, complications of pregnancy, duration of ruptured membranes, number of vaginal examinations, duration of labor, requirement for oxytocin augmentation, fetal heart rate (FHR) abnormalities, presence of meconium-stained amniotic fluid, mode of delivery, type of analgesia, febrile morbidity, and antibiotic administration. All nulliparous women were managed by a modified active management of labor protocol, as previously described.[7] Oxytocin was administered to multiparous women at the discretion of the attending physician. Continuous electronic FHR monitoring was performed in all patients. FHR abnormalities were defined according to the criteria of Freeman and Garite.[8] The diagnosis of chorioamnionitis was based on the presence of maternal temperature $\geq 38°$ C and one or more of the following: maternal tachycardia, fetal tachycardia, uterine tenderness, or malodorous amniotic fluid. Umbilical cord arterial and venous blood gases were obtained at all births at Hermann Hospital during the study period. Immediately after delivery, a segment of umbilical cord was clamped, divided, and handed to the delivery room nurse. Preheparinized 3 ml syringes (Marquest Medical Products, Englewood, Col.) were used to obtain a consistent dose and preparation of heparin. All samples were obtained in the delivery room and placed on ice. Analysis was performed within 15 minutes of delivery by a laboratory technician on a blood gas analyzer (IL-1312, Allied Co., Lexington, Mass.) located in the satellite laboratory adjacent to the labor and delivery area. Statistical analysis was performed by paired t test, multivariate Hotelling t^2 test, χ^2, and Fisher's exact test as appropriate. This was accomplished with statistical software packages (SPSS-PC, SPSS Inc., Chicago, and Abstat, Anderson-Bell, Parker, Col.). A p value of <0.05 was chosen to represent statistical significance.

Results

There were 16,345 deliveries at Hermann Hospital during the study period. Sixty-one neonates (29 term and 32 preterm deliveries) met the inclusion criteria and were matched with controls. Statistical analysis confirmed no significant differences between control and septic groups for the matched variables of age, parity, gestational age, and birth weight, respectively (Table I). There were no significant differences in racial distribution between the control and septic groups (46% black, 36% white, 18% Hispanic vs 48%, 34%, 18%, respectively). Distribution of healthcare services was 48% private health maintenance organization, 34% University of Texas clinic, and 18% private patients for each group. The cesarean section rate in this selected population was 23%. Betamethasone for fetal pulmonary maturation was given to 24% ($n = 8$) of preterm septic neonates and 20% ($n = 7$) of preterm controls ($p > 0.20$). The preterm birth rate at our institution during the study time period was 5.0%. Thus 4.3% of preterm and 0.2% of term deliveries met the criteria for sepsis acquired in utero. The incidence of in utero–acquired infection was significantly different between these groups ($p < 0.05$, odds ratio 22.4, 95% confidence limits 15.9 and 31.7). Analysis of the arterial umbilical cord gases revealed a significant decrease in pH in the septic neonates when compared with controls ($p < 0.05$). In addition, the bicarbonate and base excess were significantly lower in the septic neonates. There

Table I. Demographic characteristics

	Overall		*Term*		*Preterm*	
	Control (n = 61)	*Septic (n = 61)*	*Control (n = 29)*	*Septic (n = 29)*	*Control (n = 32)*	*Septic (n = 32)*
Age (yr)	25 ± 0.7	26 ± 0.7	26 ± 1.0	26 ± 1.0	25 ± 0.9	26 ± 0.9
Parity	1.2 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	1.5 ± 0.5	1.1 ± 0.3	1.1 ± 0.2
Gestational age (wk)	34.4 ± 0.6	34.3 ± 0.6	39.3 ± 0.2	39.1 ± 0.2	30.5 ± 0.6	30.0 ± 0.6
Birth weight (gm)	2418 ± 133	2369 ± 142	3422 ± 73	3449 ± 99	1489 ± 98	1456 ± 103

Table II. Umbilical arterial cord gas values

	pH	*P_{O_2}*	*P_{CO_2}*	*Bicarbonate*	*Base excess*
Overall					
Control (n = 61)	7.26 ± 0.06	18.0 ± 5.8	53.1 ± 9.1	24.1 ± 2.3	−2.9 ± 2.3
Septic (n = 61)	7.23 ± 0.07*	18.1 ± 6.8	54.6 ± 8.8	22.5 ± 3.1*	−4.5 ± 3.3*
Term					
Control (n = 29)	7.26 ± 0.06	18.1 ± 4.8	55.2 ± 8.7	24.7 ± 2.0	−2.7 ± 2.2
Septic (n = 29)	7.21 ± 0.07*	17.2 ± 5.4	57.6 ± 9.2	22.1 ± 3.4*	−5.0 ± 3.1*
Preterm					
Control (n = 32)	7.27 ± 0.07	18.0 ± 6.7	51.1 ± 9.1	23.5 ± 2.6	−3.3 ± 2.4
Septic (n = 32)	7.25 ± 0.07	19.0 ± 8.0	51.7 ± 7.5	22.9 ± 2.7	−3.9 ± 3.3

*$p < 0.05$, versus control.

was no alteration in arterial respiratory gas values when septic and control groups were compared. These data are summarized in Table II. Venous umbilical cord blood gases analysis also showed a significant decrease in pH in the septic cases when compared with controls ($p < 0.05$). Bicarbonate and base excess were also significantly lower in the septic neonates. Venous respiratory parameters were not altered. These data are summarized in Table III. The data were divided into preterm (<37 completed weeks of gestation) and term subsets to evaluate the effect of gestational age on cord blood gases in this population. There were no differences in umbilical arterial or venous blood gas parameters when preterm septic neonates were compared with preterm controls (Tables II and III). However, within the term subset there was a significant decrease in the arterial and venous pH ($p < 0.05$). In both arterial and venous specimens, the bicarbonate and base excess were significantly lower in the septic neonates. There were no alterations in arterial P_{O_2} or P_{CO_2} when term septic neonates were compared with controls. The respiratory component of venous gases were altered, as venous P_{O_2} was significantly lower while P_{CO_2} was significantly higher in the septic group (Tables II and III). FHR tracings were evaluated by the attending physician. The occurrence of any abnormality on FHR tracing was noted in 36% ($n = 22$) of septic and 33% ($n = 20$) of control neonates ($p > 0.20$). Two thirds of these abnormalities consisted of fetal tachycardia (>160 and <180 beats/min) or mild variable decelerations in both groups. When divided into term and preterm subsets, there were no differences in the incidence of significant FHR abnormalities (poor variability, severe variable or late decelerations) when compared with controls (10% vs 8%; $p > 0.20$). There was a significant increase in the duration of labor in the overall septic versus control group ($p < 0.05$). When preterm septic and control groups were compared, there was no significant difference in the length of labor ($p > 0.20$). However, there was a significant prolongation of labor when the term septic subset was compared with term controls ($p < 0.05$). The observed difference in the duration of labor in the overall group was accounted for by the 45% increase in the length of labor among term septic neonates. We have previously defined fetal acidemia as an arterial cord pH <2 SD from the mean of our population.[7] Using this criterion, the incidence of acidemia was significantly greater in the septic neonates compared with controls (9/61 vs 1/61, $p < 0.05$). Notably, eight of the nine acidemic neonates with cord arterial pH values of <7.14 had a duration of labor >12 hours. The control neonate with an acidemic pH had a cesarean section for presumed fetal distress after 3 hours of labor. One of the nine neonates with sepsis had a significant FHR abnormality, and elimination of this case does not change the significance ($p < 0.05$). The small numbers of patients precludes analysis of the term and preterm subsets. There was a significant in-

Table III. Umbilical venous cord gas values

	pH	Po_2	Pco_2	*Bicarbonate*	*Base excess*
Overall					
Control (n = 61)	7.34 ± 0.07	28.7 ± 7.6	42.7 ± 8.2	22.7 ± 2.1	−2.2 ± 2.2
Septic (n = 61)	7.30 ± 0.08*	27.3 ± 8.4	45.0 ± 7.7	22.0 ± 2.3*	−3.8 ± 2.8*
Term					
Control (n = 29)	7.33 ± 0.05	30.6 ± 6.1	43.7 ± 7.3	23.0 ± 1.9	−2.3 ± 1.9
Septic (n = 29)	7.27 ± 0.07*	25.2 ± 7.7*	48.1 ± 8.0*	21.6 ± 2.5*	−4.7 ± 2.8*
Preterm					
Control (n = 32)	7.34 ± 0.08	26.9 ± 8.5	41.7 ± 9.0	22.5 ± 2.1	−2.1 ± 2.2
Septic (n = 32)	7.32 ± 0.08	29.3 ± 8.5	42.4 ± 6.2	22.4 ± 2.1	−2.7 ± 1.9

*$p < 0.05$, versus control.

Table IV. Obstetric findings

	Overall		*Term*		*Preterm*	
	Control (n = 61)	*Septic (n = 61)*	*Control (n = 29)*	*Septic (n = 29)*	*Control (n = 32)*	*Septic (n = 32)*
Duration of rupture of membranes (hr)	12.5 ± 3.6	33.9 ± 8.0*	7.3 ± 4.2	11.8 ± 6.6	16.9 ± 3.1	58.7 ± 8.1*
Duration of labor (hr)	6.6 ± 0.6	8.7 ± 0.7*	7.3 ± 0.7	10.8 ± 0.9*	6.2 ± 0.8	7.0 ± 0.9
Rupture of membranes >24 hr	11% (7)	34% (21)*	7% (2)	14% (4)	16% (5)	53% (17)*
Labor >12 hr	11% (7)	31% (19)*	17% (5)	48% (14)*	6% (2)	16% (5)
Meconium	8% (5)	25% (15)*	17% (5)	31% (9)	0% (0)	19% (6)*
Chorioamnionitis	2% (1)	30% (18)*	0% (0)	17% (5)*	3% (1)	41% (13)*
Endometritis	15% (9)	28% (17)*	7% (2)	21% (6)	22% (7)	34% (11)

*$p < 0.05$, versus control.

crease in febrile morbidity among the mothers of septic neonates. Intrapartum fever (>38° C) occurred more frequently in mothers of septic newborns when compared with mothers of controls (26% vs 2%, respectively; $p < 0.05$). This febrile morbidity was evident in both the term and preterm septic subgroups when compared with controls (24% vs 0% and 28% vs 3%, respectively). The clinical diagnosis of chorioamnionitis was made significantly more often in the overall term and in the preterm septic groups when compared with controls ($p < 0.05$). In addition, there was a significant increase in the incidence of postpartum endomyometritis when mothers of septic newborns were compared with controls ($p < 0.05$) (Table IV). The duration of ruptured membranes was significantly longer in the overall septic group when compared with controls ($p < 0.05$). The preterm septic subset accounted for this difference (Table IV). In the overall group, there was a significant increase in the incidence of prolonged rupture of membranes (>24 hours) when septic neonates were compared with controls ($p < 0.05$). This difference was also attributable to the preterm subset (Table IV). There was a significant increase in the incidence of meconium-stained amniotic fluid in the overall septic group when compared with controls ($p < 0.05$). There was no statistically significant difference in the requirement for oxytocin augmentation in any of the populations studied (overall, 28% (17) vs 39% (24); term, 38% (11) vs 48% (14); preterm, 19% (6) vs 31% (10); $p > 0.20$). Similarly, there were no significant differences in regional analgesia usage (overall, 31% (20) vs 39% (24); term, 59% (17) vs 69% (20); preterm, 9% (3) vs 13% (4); $p > 0.20$). There were also no significant differences in the assignment of a 5-minute Apgar score <7 when neonates with sepsis were compared with controls in the overall, term, or preterm groups (12% vs 23%, 3% vs 13%, and 20% vs 33%, respectively; $p > 0.20$). The bacterial pathogens isolated included a variety of microorganisms, two thirds of which were group B streptococcus, *Escherichia coli* and staphylococcus species. Analysis of the isolates demonstrated a significant increase in the incidence of group B streptococcal infection in term septic when compared with preterm septic neonates (19/29 vs 11/32, respectively, $p < 0.05$). There were no differences in arterial or venous umbilical cord blood gas parameters between neonates with group B streptococcal infection compared with septic neonates with

other pathogens. One neonate in each subset had more than one pathogen identified. There were eight neonatal deaths among the septic neonates (three term, five preterm), whereas there was one neonatal death in the control group, in the preterm subgroup.

Comment

Delivery of the fetus when intrauterine infection is diagnosed or strongly suspected is currently accepted obstetric practice.[9, 10] However, no maternal[5] or neonatal[6] benefit to immediate delivery has been demonstrated with intrapartum antibiotic administration. Little is known about the relationship between in utero infection and fetal acid-base status. In this study, we have demonstrated that fetal sepsis is associated with a decrease in the umbilical arterial pH, accompanied by metabolic alterations in the arterial blood gases. These metabolic changes are evidenced by decreases in pH, bicarbonate, and base excess without alterations in arterial P_{O_2}, or P_{CO_2}. Assessment of the influence of gestational age on the arterial blood gas values indicates that the differences are accounted for by the term septic subgroup. Term septic neonates display a significant decrease in arterial pH, bicarbonate, and base excess when compared with matched nonseptic controls. Similar changes are observed in the venous umbilical cord blood gases. In the term septic subgroup, both metabolic and respiratory alterations are observed, as venous P_{O_2} is significantly decreased while P_{CO_2} was significantly elevated. This may be due to an impairment in placental function due to local infection or to a reduction in maternal perfusion in the presence of sepsis. The latter speculation is unlikely to be of significance, as only four of these women had blood culture–proved sepsis and only 30% had a clinical diagnosis of chorioamnionitis. These factors may compound the metabolic changes associated with sepsis in fetal arterial blood gases.

Curiously, cord blood gases from the preterm septic newborns are not significantly different from their nonseptic controls, in spite of the greater incidence of chorioamnionitis and prolonged rupture of the membranes in the mothers of this group. We postulate that the lack of alteration in acid-base status is likely due to the significantly shorter duration of labor in this subpopulation. This suggests that fetal sepsis with a short duration of labor does not cause significant changes in fetal acid-base status. We speculate that fetal sepsis plus prolonged labor may cause accelerated deterioration in acid-base status, as demonstrated in the term septic subgroup.

To evaluate the etiology of the observed metabolic changes in the term septic neonates, several obstetric factors were assessed. There were no significant differences found in the duration of ruptured membranes, incidence of prolonged ruptured membranes, oxytocin usage for augmentation of labor, fetal meconium passage, or regional analgesia use in the term septic versus nonseptic groups. The only obstetric factor in which term septic neonates differed from controls was in the duration of labor. A significant prolongation in the mean duration of labor was present when term septic neonates were compared with their nonseptic term controls. This significant prolongation of labor held true even when term septic neonates were compared with the preterm septic and preterm control groups. In addition, the incidence of labor duration >12 hours was significantly higher only in the term septic subset. Eight of the nine neonates with an arterial pH of <7.14 had labor >12 hours. This lends further credence to the detrimental effect of prolonged labor on acid-base balance in the presence of fetal sepsis. It is unclear from this data whether the in utero sepsis per se had a direct effect on the fetus (i.e., through lactic or other fixed acids) or whether this was a physiologic response to the increased duration of labor in the face of an already stressed septic fetus. Deterioration in fetal acid-base status with increasing duration of labor has been demonstrated previously by other authors.[11] However, as there were no differences in the length of labor when preterm septic neonates were compared with preterm controls, it is unlikely that sepsis directly causes an increase in the length of labor. Thus the observed metabolic alterations in arterial blood gas parameters appears to be attributable to the combination of fetal sepsis and increased duration of labor.

To ensure that our study population was representative of true fetal infection, we chose to assess only neonates with bacteremia documented by positive blood culture within the first 24 hours of life. These selection criteria may not include all infected neonates but do provide a study population with clearly defined sepsis. The intrapartum maternal administration of antibiotics may have rendered neonatal cultures negative in a bacteremic newborn; the exclusion of these patients from our study may have confounded the interpretation of our results. The spectrum of infectious microorganisms in this study was comparable to those of previous investigators.[12, 13] Group B streptococcal infection did not disproportionately contribute to the observed differences in arterial blood gas values. The classic maternal markers of chorioamnionitis were poor prognostic indicators of fetal sepsis. Clinical chorioamnionitis was diagnosed in only 27% of mothers of septic neonates. Features often associated with maternal antenatal sepsis, such as prolonged rupture of the membranes, duration of labor >12 hours, and postpartum endomyometritis, were present significantly more often in mothers of septic neonates than in those of nonseptic

controls. Because the majority of mothers of bacteremic neonates do not have clinical findings of chorioamnionitis, the clinical diagnosis of fetal intrauterine sepsis is extremely difficult. Additionally, the occurrence of fetal sepsis may be independent of clinical maternal infection.

Previous studies[4, 6] assessing the effect of in utero sepsis on umbilical cord blood gas parameters have focused on the diagnosis of chorioamnionitis and its impact on the fetus. Laboratory investigation revealed that the majority of these neonates were not septic. Therefore the umbilical cord blood gas values do not necessarily reflect the effect of sepsis on fetal acid-base balance. The incidence of early neonatal bacterial sepsis in our study was four in 1000 live births, in keeping with previous reports of 1 to 5 in 1000 live births.[12]

Early identification of the fetus with in utero sepsis is critical for instituting appropriate antibiotic therapy. However, the diagnosis of fetal sepsis is difficult, with recognizable chorioamnionitis being present in only the minority of cases. A high index of suspicion remains the major diagnostic criterion, with prolonged rupture of membranes and increased duration of labor being major risk factors. The 22-fold increase in the risk of bacteremia at birth in preterm versus term neonates may be due to a number of factors. These include ascending infection as the cause of preterm premature rupture of the membranes and the preterm delivery and the significant increase in the duration of membrane rupture in preterm neonates.

In our study fetal sepsis at term was accompanied by a statistically significant reduction in arterial and venous pH. The metabolic alterations in the arterial blood gases are likely due to lactic acidemia secondary to the infection. Although of statistical significance, these differences remain well within the previously reported normal range at our institution[14] and may not be clinically significant. Our data suggest that acidemia at birth is a poor predictor of fetal sepsis, as >85% of septic neonates had a normal cord pH. Additionally, these data confirm the findings of Ramin et al.[15] that neither fetal nor maternal sepsis is an indication for cesarean section.

The clinical significance of these findings lies in the increased incidence of fetal acidemia in septic neonates and in the association between increased duration of labor and fetal sepsis. From our study it is unclear whether fetal sepsis can contribute to a prolongation of labor or whether prolonged labor leads to fetal sepsis. However, our findings lend support to the concepts of active management of labor, which have been demonstrated to reduce the duration of labor.[16] In our institution, application of these principles has decreased the duration of labor without altering fetal acid-base status.[7]

There are four major conclusions that can be drawn from our study: (1) Fetal sepsis with short duration of labor does not cause significant changes in acid-base status; (2) fetal sepsis alone does not cause an increase in the duration of labor; (3) there is, however, an association between the duration of labor, fetal sepsis and fetal acidemia at term; (4) preterm neonates have a significantly increased risk of fetal sepsis when compared with term neonates. Further study of the potential clinical effects of fetal sepsis is indicated.

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Eradication of *Ureaplasma urealyticum* from the amniotic fluid with transplacental antibiotic treatment

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Ureaplasma urealyticum was isolated from the amniotic fluid of a patient with preterm premature rupture of membranes at 24 weeks. A second amniocentesis performed 48 hours later indicated an increase in the number of neutrophils in the amniotic fluid. Treatment with erythromycin, ampicillin, gentamicin, and clindamycin was instituted for a period of 6 days. Amniotic fluid analysis from a third amniocentesis performed 24 hours after discontinuation of antibiotic treatment revealed only a few white blood cells and was negative for microorganisms. The pregnancy continued for 22 days after admission, at which time spontaneous labor began. The neonate survived with no sequelae and had negative blood cultures. Antibiotic treatment was associated with eradication of *Ureaplasma urealyticum* from the amniotic cavity, pregnancy prolongation, and neonatal survival. (AM J OBSTET GYNECOL 1992;166:618-20.)

Key words: *Ureaplasma urealyticum*, chorioamnionitis, antibiotic treatment, prematurity, premature rupture of membranes

A growing body of evidence suggests an association between subclinical intraamniotic infection and preterm delivery. *Ureaplasma urealyticum* is one of the most frequently isolated microorganisms from the amniotic fluid of women in preterm labor with intact membranes and from women with preterm premature rupture of membranes.[1] This microorganism has been implicated in the etiology of both maternal and fetal-neonatal infections.

The current standard management of a pregnancy complicated by microbial colonization of the amniotic cavity, as detected by transabdominal amniocentesis, has been induction of labor to prevent the maternal and fetal consequences of infection. In women with preterm gestations (i.e., <26 weeks), this approach often results in significant perinatal morbidity and mortality because of extreme prematurity. There has recently been interest in alternative management schemes to this problem, including antibiotic administration, with the aim of eradicating microbial colonization and preventing the development of infection.[2]

The purpose of this article is to report the successful eradication of *U. urealyticum* colonization of the amniotic cavity by maternal treatment with erythromycin.

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Case report

A 26-year-old woman, gravida 4, para 0, spontaneous abortions 2, elective abortions 1, was admitted to Yale–New Haven Hospital because of preterm premature rupture of membranes at 24 weeks' gestation. A sterile speculum examination was positive for an amniotic fluid pool in the vagina, ferning, and a Nitrazine test. The maternal temperature was 99.2° F, and the white blood cell count was 11,400/mm³. The differential count was as follows: segmented cells, 68%; bands, 13%; lymphocytes, 14%; and monocytes, 5%. A sonogram showed a single fetus in vertex presentation and a decreased amniotic fluid volume. Fetal biometry was consistent with 24 weeks of gestation, and the estimated fetal weight was 760 gm. Amniotic fluid, retrieved by transabdominal amniocentesis, showed the presence of white blood cells (+1), but no bacteria were found by Gram stain examination. Amniotic fluid was cultured for aerobic and anaerobic bacteria and for *Mycoplasma* sp. with the Mycotrim system (Mycotrim-GU, Berkeley, Calif.). Forty-eight hours later, the amniotic fluid culture was reported to be growing *U. urealyticum*. There was no clinical evidence of chorioamnionitis, and the white blood cell count was 9600/mm³. The differential count showed segment cells, 51%; bands, 32%; lymphocytes, 12%; and monocytes, 5%. An amniocentesis was repeated to confirm the diagnosis and for evaluation of amniotic fluid. The number of white blood cells observed increased dramatically (+4), but still no microorganisms were visualized by Gram stain examination.

The patient was started on a regimen of erythromycin (1 gm, every 6 hours, intravenously), clindamycin (900 mg, every 8 hours, intravenously), gentamicin (80 mg, every 8 hours, intravenously), and ampicillin (2 gm, every 4 hours, intravenously).

Erythromycin was chosen to cover *U. urealyticum,* and the other antibiotics were added to broaden the spectrum of coverage for other microorganisms that might not have been identified because of their fastidious nature. Four days after the second amniocentesis, the laboratory confirmed that the culture of amniotic fluid grew *U. urealyticum.* Antibiotic treatment was continued for 6 days. Twenty-four hours after discontinuation of antibiotic treatment, a third amniocentesis was performed. There were only a few white blood cells, and the Gram stain examination revealed no microorganisms. Amniotic fluid culture was negative for bacteria and *Mycoplasma* sp.

The patient remained asymptomatic for two additional weeks. At that time she complained of vaginal bleeding and went into labor. A repeat amniocentesis was declined. A 1090 gm male neonate was born spontaneously at 27 weeks 3 days of gestation with Apgar scores of 1 at 1 minute and 5 at 5 minutes. The cord pH was 7.28. The neonate required intubation and mechanical ventilation. All neonatal cultures were negative. Four days after birth, the newborn was extubated. Two weeks later, the infant was transferred to another nursery. Follow-up 1 year later shows that the infant has experienced normal neurologic and intellectual development. Histopathologic examination of the placenta demonstrated severe inflammation, suggesting that chorioamnionitis had occurred. There was no evidence of puerperal endometritis.

Comment

This case represents the first report of eradication of *U. urealyticum* colonization of the amniotic fluid with maternal antibiotic therapy. This management approach was associated with continuation of pregnancy for 22 days after the amniocentesis and the birth of a more mature preterm infant.

U. urealyticum is present in the genital tract in as many as 80% of women with normal pregnancy. The mere presence of this microorganism in the genital tract is not a risk factor for preterm delivery. However, *U. urealyticum* is frequently isolated from the amniotic fluid of women in preterm labor, with or without intact membranes.[1]

Once microbial invasion of the amniotic cavity has been established by analysis of fluid retrieved by transabdominal amniocentesis, the standard management approach has been expeditious delivery. This approach has evolved to prevent maternal and fetal infection. The implicit premise is that the natural history of microbial colonization of amniotic fluid is to progress toward infection. Data obtained from patients with preterm premature rupture of membranes who underwent serial amniocenteses indicate that the inoculum size increases with time in patients with a positive amniotic fluid culture, and this is eventually associated with the onset of labor.[1] More data are required to establish whether the natural history of *U. urealyticum* colonization of the amniotic fluid is similar to that described for bacteria.

In this patient's case we were faced with three alternative management schemes: (1) to adopt the traditional approach and induce labor, (2) to observe the patient and await the spontaneous onset of parturition or the development of clinical chorioamnionitis, or (3) to attempt to eradicate colonization of the amniotic cavity. Because of the extreme prematurity of the fetus, we believed that induction of labor would have significant risk for the fetus. Although expectant management could be defended as a reasonable approach, our experience with microbial colonization in the setting of premature rupture of membranes has been that spontaneous onset of labor, and often infection, develops in a relatively short period of time.[1] Since our group had previously successfully eradicated amniotic fluid colonization and prolonged pregnancy in a case of preterm premature rupture of membranes with a mixed bacterial infection, this approach had considerable appeal.[2]

Our choice of antimicrobial therapy deserves comment. In vitro studies of the antimicrobial sensitivity of *U. urealyticum* have shown that up to 90% of strains are sensitive to erythromycin. However, in vivo studies indicate that this antibiotic is ineffective in eradicating *U. urealyticum* from the lower genital tract. This discrepancy between in vitro and in vivo data has been attributed to the effect of the vaginal pH, since erythromycin is a less effective antimicrobial agent at low pH. Because erythromycin crosses the placenta and reaches the amniotic cavity and the amniotic fluid had a neutral pH, we believed that this antimicrobial agent would be the best choice. We chose to add the other antibiotics to cover fastidious microorganisms that may not have been recovered before initiation of this therapy.

Although the amniotic cavity was sterile after discontinuation of antibiotic therapy, it is unknown if microbial colonization recurred before delivery because the patient declined an amniocentesis at the time of onset of preterm labor. In this case neonatal cultures were negative, indicating that if colonization of the amniotic cavity had recurred, it had not caused a systemic neonatal infection. Histopathologic examination of the placenta suggested acute chorioamnionitis; therefore it is tempting to postulate that the failure of host-defense mechanisms that led to the original colonization of the amniotic cavity allowed recolonization once antibiotic treatment was discontinued. A small separation of the placenta may have been responsible for the vaginal bleeding and may also have contributed to the initiation of preterm labor.[3]

This case demonstrates that antibiotic administration to the mother is a reasonable option for the management of colonization of the amniotic cavity in cases of

extremely preterm gestations. In our case prolongation of pregnancy was accomplished without mishap to the mother or the fetus.

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Vaginal Po_2 in healthy women and in women infected with *Trichomonas vaginalis*: Potential implications for metronidazole therapy

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We measured the Po_2, pH, and temperature in the vaginal canals of nine patients with symptomatic *Trichomonas* vaginitis and those of 10 healthy women. The patients included eight women with primary infections caused by metronidazole-susceptible strains and one refractory case that resulted from infection with a metronidazole-resistant *Trichomonas vaginalis*. The median vaginal Po_2, pH, and temperature in the patient group were 1 mm Hg, 6, and 37.3° C respectively; these medians were 1 mm Hg, ≤4.5, and 37.2° C in the healthy group. These data show that vaginal environment is anaerobic or microaerophilic (it has reduced oxygen tension). Because the activity of metronidazole is reduced under aerobiosis, the vaginal environment should enhance the biologic activity of the drug. (AM J OBSTET GYNECOL 1992;166: 620-4.)

Key words: Vaginal Po_2, metronidazole, trichomoniasis

The activity of antimicrobial agents is influenced by local factors that reside at the infected site.[1, 2, 3] This is relevant to the vaginal canal, in which the in vitro activity of metronidazole against *Trichomonas vaginalis* is shown to be enhanced when tests are performed under anaerobic conditions.[4, 5] Increased activity of metronidazole under anaerobiosis is most noticeable when metronidazole-resistant isolates are examined.[6, 7] This report defines the physiologic parameters of the vaginal environment with particular emphasis on Po_2 in two groups of women: patients with vaginal infection caused by *T. vaginalis* and healthy women not infected with *T. vaginalis*. Vaginal Po_2 was measured with a transcutaneous O_2 monitor that uses a miniature Clark electrode (Novametrix Medical Systems, Inc., Wallingford, Conn.)

Material and methods

Subjects studied included nine patients suffering from symptomatic *Trichomonas* vaginitis documented by positive wet mount and culture and 10 healthy women without vaginitis in whom wet mount and culture were negative for *T. vaginalis*. Research protocol was ap-

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Table I. Microbiologic and physiologic measurements in the vaginal canal of trichomoniasis patients and healthy women

	Trichomoniasis patients $n = 9$	*Healthy women* $n = 10$
Age		
Mean	28	33
Range	21-35	28-38
Prior hysterectomy	2/9	0/10
Wet mount for clue cells	0/9	0/10
Wet mount for *Trichomonas vaginalis*	9/9	0/10
Culture for *Trichomonas vaginalis*	9/9	0/10
Culture for *Candida*	1/9*	0/10
Vaginal temperature (degrees Centigrade)		
Median	37.3	37.2
Range	36.9-37.7	36.7-37.5
Vaginal pH		
Median	6	≤4.5
Range	5-6.5	≤4.5-6.5
Vaginal Po_2 (mm Hg)		
Median	1	1
Range	0-13	0-28

*Culture from one patient had 1+ *Candida albicans*.

proved by the institution's Committee on Human Research, and each participant signed a consent form before the investigation was conducted. Wet mounts of vaginal secretions were examined for *T. vaginalis*, clue cells, and yeast or hyphae. In addition, fungal cultures for *Candida* were performed. Cultures for *Trichomonas* were performed with modified Diamond's medium (which incorporates vancomycin, 20 μg/ml; gentamicin, 20 μg/ml, and amphotericin B, 10 μg/ml instead of the penicillin G and streptomycin used in the original formula) and Feinberg-Whittington medium. The vaginal pH was measured by exposing a nitrazine paper strip (E.R. Squibb and Sons, Inc., Princeton, NJ) to the vaginal secretions. The scale on the paper strip ranged from pH 4.5 to pH 7.5. The vaginal Po_2 was measured with a transcutaneous O_2 monitor 811 (Novametrix Medical Systems, Inc., Wallingford, CT), which uses a miniature Clark electrode. The instrument was calibrated according to the manufacturer specifications. The high Po_2 was set at the calculated partial pressure of atmospheric oxygen (corrected for water vapor pressure) and zero Po_2 was set at zero during the application of the manufacturer's zero Po_2 solution. The electrode was further checked against solutions gassed with different mixtures of oxygen and was measured with a Radiometer BMS3 Mk2 (Radiometer, Copenhagen, Denmark) blood gas analyzer that is calibrated with commercial gas mixtures. In test solutions, the electrode was always within 2 mm Hg of the blood gas electrode, even at high Po_2 (about 150 torr). Thereafter, the probe was inserted into the vagina without the introduction of ambient air and was left in place until a constant reading was obtained for at least 2 minutes. Vaginal Po_2 was measured first; this was followed by the collection of specimens for wet mount examination, culture, and pH measurement. None of the women in the healthy or *Trichomonas* groups were menstruating when Po_2 was measured. Metronidazole in vitro susceptibility tests were performed by means of microdilution and macrodilution broth methods on *T. vaginalis* isolates from eight patients. The procedure used was a modification of that described by Meingassner and Thurner.[4] Susceptibility tests were performed at 37° C under aerobic and anaerobic conditions of incubation. Minimal inhibitory concentration (MIC) was recorded as the lowest drug concentration that resulted in complete inhibition of motility of trichomonad cells. Each variable was summarized by calculating the median and range for each group. The two groups (patients with trichomoniasis and healthy controls) were compared on each variable with the Mann-Whitney test.

Results

Table I summarizes the data from nine trichomoniasis patients and 10 healthy women. Two of the patients (one of whom represented a primary infection and the second, a refractory infection) had had hysterectomies. Eight of the nine patients had primary infections. Seven were cured by the standard metronidazole therapeutic regimen (either single-dose or multidose). The eighth patient could not be treated because of severe allergy to metronidazole. Isolates from seven of these eight patients were available for in vitro susceptibility testing. The median (range) aerobic and anaerobic MICs were 2.5 μg/ml (1.25 to 10 μg/ml) and 1.25 μg/ml (0.6 to 2.5 μg/ml), respectively. The refractory patient was a 34-year-old woman who had had persistent infection for the previous 15 months in spite of attempts on two occasions to achieve a cure with the multidose oral metronidazole regimen. Factors

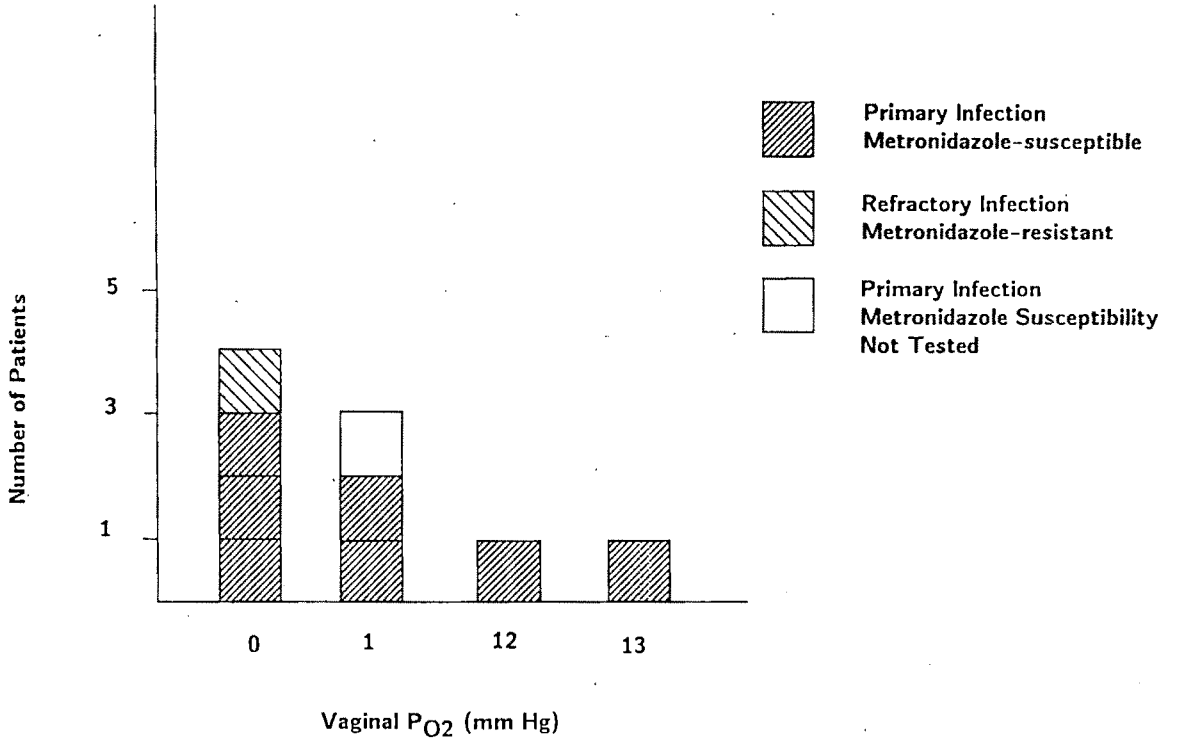


Fig. 1. Vaginal P_{O_2} in nine patients infected with *Trichomonas vaginalis*.

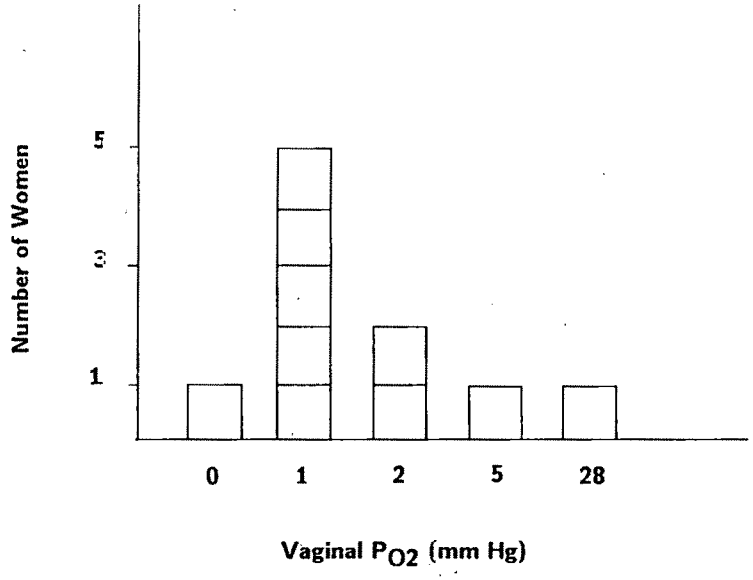


Fig. 2. Vaginal P_{O_2} in 10 healthy women.

such as reinfection and poor compliance (ruled out by measuring metronidazole blood levels) were excluded as contributions to therapeutic failure. The median aerobic and anaerobic MICs for the isolate recovered from this patient were ≥160 μg/ml and 2.5 μg/ml, respectively. The vaginal P_{O_2} was measured on two different occasions in this refractory patient, and the results were 0 mm Hg and 1 mm Hg. The median number of days between P_{O_2} measurement and onset of last menstruation was 16 days in the patient group (range 8 to 28 days) and 12 in the healthy control group (range 7 to 30 days). There was no difference in the median vaginal P_{O_2} values in the patient or control groups ($p = 0.23$). There also was no difference in the vaginal

temperatures of women in both groups ($p = 0.21$) or in the number of days between Po_2 measurement and the onset of last menstruation ($p = 0.22$). There was a tendency for the vaginal pH values to be higher in the patients compared with those of the controls; the respective median values were 6.0 and ≤4.5 ($p = 0.07$). Figures 1 and 2 illustrate the distribution of vaginal Po_2 measurements in the patient group and the healthy women groups.

Comment

Local factors residing at the infected site are known to affect the activity of antimicrobial agents.[1, 2, 3] Conditions in vitro, such as medium composition and atmospheric condition of incubation, influence the MIC and minimal lethal concentrations of antimicrobial agents.[8, 9] Activity of metronidazole against *T. vaginalis* is enhanced by anaerobiosis. Aerobic MICs tend to be higher than do anaerobic MICs. This is most noticeable in metronidazole-resistant strains recovered from refractory cases.[10, 11, 12] In 1979 Meingassner and Thurner[4] were first to isolate a metronidazole-resistant *T. vaginalis* strain from a patient afflicted with recurrent symptomatic trichomoniasis. The aerobic and anaerobic minimal lethal concentrations were 100 and 0.4 μg/ml, respectively. Before that time, refractory cases of trichomoniasis that failed to respond to metronidazole therapy were usually attributed to factors such as reinfection from male consorts, poor compliance, or inadequate drug levels that were due to poor absorption and/or inactivation or competition for drug by vaginal microbial flora.

Currently, no standardized test is available for determining in vitro susceptibility of *T. vaginalis* to metronidazole. Nevertheless, the test described by Meingassner and Thurner[4] or one of its modifications[5] has been successfully used to identify metronidazole resistance. To discriminate between metronidazole-susceptible and metronidazole-resistant strains, the test must be performed under aerobic conditions of incubation. Our study shows that the vaginal environment is anaerobic or microaerophilic (it has reduced oxygen tension). The vaginal Po_2 was ≤2 mm Hg in all but four of the 19 women (two healthy women and two patients) evaluated. Studies by Holmes et al.[13] demonstrated a reduced vaginal redox potential in bacterial vaginosis. The redox system was in a more reduced state in women with bacterial vaginosis than in controls. Successful treatment with metronidazole resulted in a decrease in vaginal pH that was accompanied by an increase in reduced vaginal redox potential. Wagner and Ottesen,[14] in their study of vaginal physiology during menstruation measured the Po_2 in a group of young healthy women. Mean Po_2 in the menstrual fluid on day 2 (in four women studied) and on day 4 (in three women) after the onset of menstruation were 52 and 46 mm Hg, respectively. These investigators also measured the vaginal Po_2 and demonstrated a wide range of values from 0 to 77 mm Hg. They attributed the variation in Po_2 to differences in the release of menstrual blood from the cervical canal into the vagina. It is also possible that the high Po_2 values observed in some of the women in that study may have resulted from obtaining the measurements after the menstrual fluid had been collected and a speculum had been inserted to obtain samples for culture. None of the women who participated in our study was menstruating when measurements were obtained. Furthermore, special care was taken to avoid introduction of air into the vagina before Po_2 was measured. Samples for cultures were collected after Po_2 was measured.

The factors responsible for maintaining a low Po_2 in the vaginal canal are not known, but this may be due to activity of the vaginal microbial flora. The microbicidal effect of metronidazole on *Trichomonas* results from reductive activation of the drug, which yields cytotoxic short-lived reduced intermediates.[15] An anaerobic environment or an environment of reduced oxygen tension within the vaginal canal should enhance the biologic activity of metronidazole. A low Po_2 in the vagina should raise the question of whether in vitro testing in aerobic conditions reflects the degree of in vivo resistance. Lossick et al.[6] have recently recognized that MIC and minimal lethal concentration measurements derived from tests performed in air tend to overestimate the degree of in vivo resistance. These authors and others cured more than 85% of refractory trichomoniasis cases that resulted from infection with metronidazole-resistant strains by administering metronidazole in doses much larger than those of the standard regimen; this suggests that resistance is relative.[6, 16, 17]

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Immunoreactive circulating endothelin-1 in normal and hypertensive pregnancies

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OBJECTIVE: The purpose of this study was to measure the circulatory levels of endothelin-1 in the serum of pregnant women with hypertension.
STUDY DESIGN: Endothelin-1 levels were measured by means of radioimmunoassay in the serum of 26 pregnant women with hypertension (14 with pregnancy-induced preeclamptic toxemia, 12 with chronic hypertension) and in the serum of 17 control pregnant women and 18 control nonpregnant women. The mean levels in the different groups were subject to statistical analysis with the analysis of variance.
RESULTS: The mean level among the women with preeclampsia (29.9 ± 13.2 fmol/ml) was significantly higher than those of the chronically hypertensive women (16.1 ± 7.3 fmol/ml, $p = 0.002$) and of the control pregnant women (19.7 ± 9.2 fmol/ml, $p = 0.011$). The mean level of the control nonpregnant women (26.9 ± 9.3) was significantly higher than that of the control pregnant women ($p = 0.029$). Among the patients with preeclampsia there was no correlation between endothelin-1 levels and the mean arterial blood pressure. Six to 10 weeks after delivery the mean levels of 15 studied patients (7 with preeclampsia, 8 with chronic hypertension) were similar to the levels of the nonpregnant control women.
CONCLUSION: We conclude that increased endothelin-1 production may play a role in the pathogenesis of preeclampsia. (AM J OBSTET GYNECOL 1992;166:624-8.)

Key words: Endothelin-1, preeclampsia

Endothelin-1, a 21-amino-acid peptide recently discovered by Yanagisawa et al.,[1] is the most potent natural pressor substance known. The messenger ribonucleic acid for endothelin-1 has so far been detected only in endothelial cells. Certain substances, including thrombin and epinephrine, reportedly cause the in vitro release of endothelin-1 from endothelial tissue.[2] Al-

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though endothelin-1 is rapidly eliminated from the bloodstream,[3,4] its intravenous injection in animals produces diffuse vasoconstriction and relatively prolonged arterial hypertension.[5,6] High levels of endothelin-1 have been detected in the sera of patients with essential hypertension[7] and acute renal disorders,[8,9] whereas endothelin-1 activity was recently shown to interact with vasoactive prostaglandins.[10-12] Therefore one might expect endothelin-1 to play a role in the pathophysiologic composition of preeclamptic toxemia, a disorder characterized by hypertension in pregnancy, impaired renal function, systemic vasospasm, and altered production of vasoactive prostaglandin in platelets and endothelial tissue. The purpose of this study was to compare the activity of endothelin-1 in hypertensive pregnant women in general with those in normal pregnant and nonpregnant women. Because it is assumed that chronic hypertension in pregnancy and preeclampsia are distinct entities, we compared particularly the activity of endothelin-1 among these two groups of pregnant women.

Patients and methods

Patients. The study group consisted of 26 women, between 20 and 39 years old, in the third trimester of pregnancy, who were admitted to our department over a 3-month period because of pregnancy-induced hypertension, i.e., systolic blood pressure >140 mm Hg or diastolic pressure >95 mm Hg at least twice within 6 hours. The disappearance (phase V) Korotkoff sound was used to define diastolic blood pressure. Twelve of the patients had a history of hypertension before the twentieth week of the current pregnancy and were accordingly designated as chronically hypertensive. None of these patients had proteinuria of >0.5 gm/24 hours on admission. The other 14, all of them nulliparous with no history of hypertension before the third trimester, were conisdered to have preeclampsia. Nine of these patients had proteinuria of >0.5 gm/24 hours on admission. We also classified the women, whether chronically hypertensive or preeclamptic, according to the severity of the hypertensive disorder; in those with systolic blood pressure <160 mm Hg, diastolic blood pressure <110 mm Hg, and proteinuria of ≤+1, the disorder was considered to be mild to moderate, whereas in those with higher systolic or diastolic blood pressure or proteinuria of ≥+2 it was classified as severe. Within 24 hours of admission, brachial venous blood (10 ml) was withdrawn from each patient, none of whom was yet in spontaneous or induced active labor.

Two control groups consisted of 35 women aged between 20 and 39 years who were randomly chosen over the same period. The first consisted of 17 pregnant women in the third trimester of pregnancy, all of whom had normal blood pressure and no previous history of hypertension. The second consisted of 18 healthy, nonpregnant women with no history of essential hypertension or previous hypertension in pregnancy. Venous blood samples were withdrawn from all the women in the control groups.

The women in the study group and in the first and second control groups were similar with respect to both mean age (27.4 ± 5.5, 28.8 ± 3.8, and 27.9 ± 4.1 years, respectively) and parity (1.9 ± 0.7, 1.5 ± 0.6 and 1.9 ± 0.9, respectively). Six to 10 weeks (median 57 days) after delivery we reexamined 15 of the patients in the study group. After determining that the preeclamptic women were no longer hypertensive and had no proteinuria and that the chronically hypertensive women had blood pressure values similar to those before being pregnant, we remeasured the venous blood endothelin-1 levels.

Methods. Blood was collected in tubes containing 7.5 mmol/L ethylenediaminetetraacetic acid and aprotinin (500,000 IU/ml), centrifuged immediately at 4° C, and stored at −20° C until assayed. After acidification and passage through Amprep 500 mg C_2 columns (Amersham Int., England), endothelin-1 levels in the serum were determined by means of the iodine 125–labeled endothelin-1,2 assay system (Amersham), with a sensitivity of 1.03 fmol per tube, an intrassay coefficient of variation of 4.1%, and an interassay coefficient of variation of 13%. Cross-reactivity with endothelin-3 was 0.0024%.

The results and data regarding patients characteristics are reported as the mean ± SD. Statistical comparisons between the groups were carried out by means of the analysis of variance test. Comparisons between patients' endothelin-1 levels before and after delivery were carried out by means of the adjusted paired Student t test. Differences were considered significant if the p value was <0.05. The correlation between mean arterial pressure (Mean arterial pressure = ⅔ Diastolic pressure + ⅓ Systolic pressure) and endothelin-1 levels was determined according to univariate linear regression and the Pearson coefficient of correlation.

Results

The serum endothelin-1 levels of the women in the different groups are shown in Fig. 1. The mean level in the preeclamptic study subgroup (29.9 ± 13.2 fmol/ml) was significantly higher than that of both the chronically hypertensive study subgroup (16.1 ± 7.3 fmol/ml, $p = 0.002$) and the normal pregnant control group (19.7 ± 9.2 fmol/ml, $p = 0.011$) and similar to the mean level in the nonpregnant control group (26.9 ± 9.3 fmol/ml). The mean level in the nonhypertensive pregnant control group was significantly lower than that of the nonpregnant control group ($p = 0.029$) and similar to that of the chronically hy-

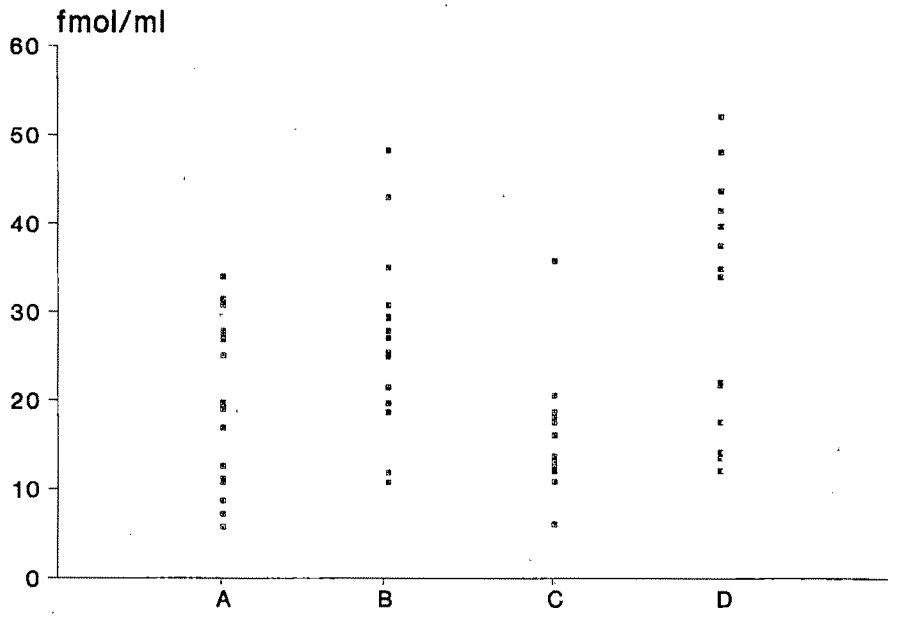


Fig. 1. Serum endothelin-1 levels of women in different groups. **A,** Normal pregnant controls; **B,** nonpregnant controls; **C,** chronic hypertensive; **D,** preeclamptic.

pertensive study subgroup. Within the two study subgroups we found no significant differences between mean serum endothelin-1 levels of patients with severe hypertensive disorder compared with those with a mild to moderate one; five patients with severe chronic hypertension (average blood pressure >160 mm Hg systolic or 110 mm Hg diastolic) had a mean endothelin-1 level similar to that of the other seven mild to moderate chronically hypertensive patients (14.9 ± 11.8 and 14.9 ± 4.7 fmol/ml, respectively). Eight severely preeclamptic patients who had average blood pressure >160 mm Hg systolic or 110 mm Hg diastolic ($n = 6$) or proteinuria of ≥+2 ($n = 7$) had a slightly but not significantly higher mean endothelin-1 level compared with that of the other patients with mild to moderate preeclampsia (32.3 ± 13.3 and 28.9 ± 15.2 fmol/ml, respectively).

The mean arterial pressure (when blood samples for endothelin-1 measurements were withdrawn) was 117.7 ± 11.1 mm Hg for the chronically hypertensive patients and 116.2 ± 11.9 mm Hg for the preeclamptic patients. No correlation between circulatory endothelin-1 levels and mean arterial blood pressure was found for all the study group ($n = 26$, $r^2 = 0.09$) and when the correlation test was restricted to the preclamptic patients only ($n = 14$, $r^2 = 0.19$).

Fig. 2 compares the serum endothelin-1 levels of 15 study group patients measured 6 to 10 weeks after delivery, when the patients were normotensive, with their levels during the acute hypertensive phase before delivery. The mean endothelin-1 level of the eight chronically hypertensive women of the study group (study subgroup 1) had significantly increased from 13.2 ± 3.7 to 21.6 ± 8.7 fmol/ml ($p = 0.05$), whereas that of the seven preeclamptic women of the study group (study subgroup 2) had slightly but not significantly increased from 21.2 ± 4.6 to 27.2 ± 5.8 fmol/ml ($p = 0.07$). The new mean levels after delivery were now similar to that of the nonpregnant control patients (26.9 + 9.3 fmol/ml).

Comment

Preeclamptic toxemia of pregnancy is a major cause of obstetric and perinatal morbidity.[13] Its pathophysiologic composition, however, is not well understood. Some authors have suggested that the primary abnormality of this disorder is an aberrant platelet metabolism,[14] with overproduction of vasoactive prostaglandins[15] and consequent endothelial damage and increased peripheral vascular resistance. The resulting symptoms are increased systemic blood pressure, alterations in renal function, and activated coagulation state.

Because the recently discovered endothelium-derived peptide endothelin-1 is a highly potent vasoconstrictor and because elevated levels have already been implicated as a possible factor in essential hypertension,[7] acute renal failure,[8, 9] and platelet hyperreactivity,[16] we were interested in investigating the possible role of endothelin-1 in the etiology of preeclampsia. Two recent reports by Kamoi et al.[17] and Taylor et al.[18] demonstrated relatively high levels of endothelin-1 in a small group of women with pregnancy-induced hypertension, as determined by means of sandwich-type

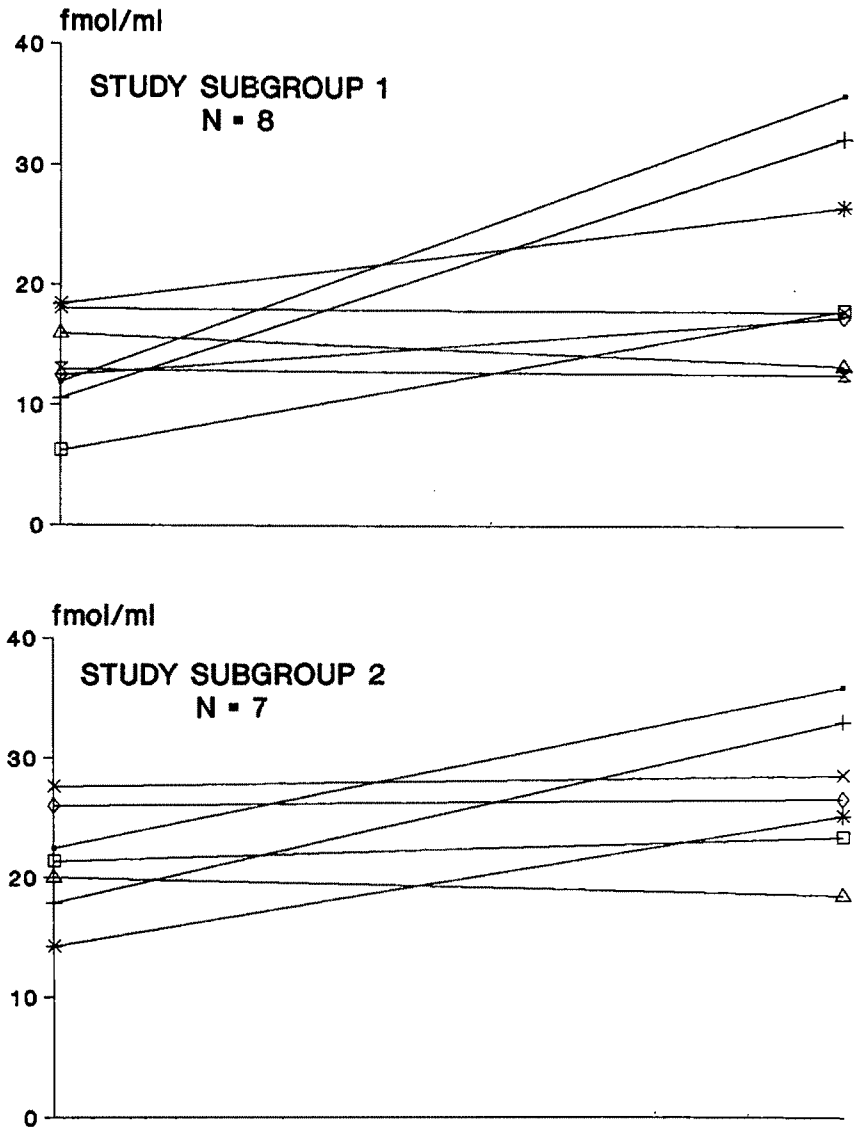


Fig. 2. Serum endothelin-1 levels of 15 study group patients measured 6 to 10 weeks after delivery compared with their levels during acute hypertensive phase before delivery. *Study Subgroup 1*, Chronically hypertensive women. *Study Subgroup 2*, Preeclamptic women.

enzyme immunoassay and radioimmunoassay kit, respectively. Our findings are in accordance with these recently reported results and suggest that serum levels of endothelin-1 are significantly decreased during normal pregnancy and that preeclamptic toxemia of pregnancy is associated with serum levels that are significantly higher than those in a normal pregnancy. However, we could not demonstrate, in our group of preeclamptic patients, a correlation of the circulating endothelin-1 levels to the severity of the disorder.

High levels of circulating endothelin-1 may reflect increased synthesis or reduced elimination of the peptide. An increase in endothelial production in patients with preeclamptic toxemia might be a feedback-related outcome of interaction with prostacyclin and endothelium-derived relaxing factor synthesis.[10-12, 19, 20] Thus the more disturbed the prostaglandin and platelet metabolism, the more increased the synthesis and release of endothelin-1 by endothelial cells. Such a feedback mechanism might be regulated by signals derived from intracellular calcium stores and protein kinase C activation, a recently proposed cellular action site of endothelin-1.[21] Because endothelin-1 is produced from preproendothelin, the activity of the converting enzyme that controls the release of the immunoreactive endothelin-1 peptide and the expression of the preproendothelin-1 messenger ribonucleic acid may be targets for the proposed feedback mechanism. Alternatively, the differences in levels of circulating endoth-

elin-1 might be explained by a structural or functional alteration in the vascular receptors for this peptide, which has already been identified in the human placenta.[22]

The basic pathophysiologic characteristics of chronic hypertension in pregnancy are obviously different from those of preeclamptic toxemia and probably more closely resemble those of essential hypertension. Our findings regarding the low endothelin-1 levels of the chronically hypertensive pregnant patients are not in accordance with the relatively high circulatory endothelin-1 levels reported by others in nonpregnant patients with essential hypertension. We thus assume that the pregnancy-reducing effect on the endothelin-1 synthesis contradict and conceal the hypertensive effect in cases of chronic hypertension in pregnancy. It should be noted that although the patients designated here as chronically hypertensive were admitted because of an aggravated hypertensive state during pregnancy, their low endothelin-1 levels resemble those of the normal pregnant patients and not those of the patients designated as preeclamptic. These results accentuate the differences between chronic hypertension and preeclamptic hypertension in pregnancy and may reflect the possible role of endothelin-1 in the pathogenesis of the systemic vascular abnormality of the preeclamptic syndrome.

The results of this preliminary study show that circulatory immunoreactive endothelin-1 levels decline during normal pregnancy relative to their levels in nonpregnant women but increase significantly during preeclamptic illness. Enhanced endothelin-1 production may be associated with the etiology of preeclampsia or rather may be the result of local hormonal feedback mechanisms involving the endothelium.

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Platelet activation and vascular damage in gestational hypertension

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Increased plasma fibronectin levels are a highly sensitive and specific predictor of gestational hypertension. Of a total of 105 apparently healthy normotensive primigravid women seen at the outpatient clinic, 10 with increased plasma levels of fibronectin (mean ± 2 SD), were compared with 14 controls. Parameters of early vascular damage (laminin, preprocollagen III), platelet activation (β-thromboglobulin, platelet factor 4), and coagulation (thrombin–antithrombin III complexes, fibrinopeptide A) were measured at regular (weekly or monthly) intervals. Abnormal values of laminin ($p < 0.005$) and fibronectin ($p < 0.0001$) were found up to 4 weeks before the onset of clinical disease. Levels of β-thromboglobulin ($p < 0.0001$) were also elevated at least 4 weeks before the appearance of clinical symptoms. Our results show that increased levels of laminin, fibronectin, and platelet activation, as indicated by β-thromboglobulin levels, are preclinical features of gestational hypertension and indicate that vascular damage has occurred. Fibrin formation would appear to occur later. (Am J Obstet Gynecol 1992;166:629-33.)

Key words: Fibronectin, laminin, β-thromboglobulin, preeclampsia

The etiology of hypertensive disease in pregnancy remains enigmatic. A variety of different pathogenic pathways may lead to hypertension, which in some cases is associated with chronic consumptive coagulopathy. Endothelial and subendothelial cell damage may induce platelet activation or may directly liberate tissue factor,[1] a cofactor initiating the extrinsic coagulation pathway. The inability to limit the extent of platelet activation, as a result of a reduction of the prostacyclin-to-thromboxane A_2 ratio[2] and a reduction of the fibrinolytic capacity, may aggravate the continuing coagulopathy.[3] Another blood platelet containing vasoactive substances such as serotonin have a dramatic influence on blood pressure.[4]

Fibronectin, laminin, and preprocollagen III are parameters related to endothelial cell damage. Fibronectin, an adhesive glycoprotein, is produced by fibroblasts, endothelial cells, macrophages, and thrombocytes[5] and is a widespread component of connective tissue and basement membranes. It is involved in many functions, such as cell adhesion, cell motility, tissue repair, opsonization, and coagulation.[6] Increased plasma fibronectin levels in pregnant women predict the development of gestational hypertension with a sensitivity and specificity of 95% and precede the onset of clinical symptoms, on average, by 4 weeks.[7, 8] Laminin is synthesized by endothelial cells and is a major intrinsic component of the basement membrane. Preprocollagen III is a marker for both synthesis and mobilization of type III collagen, a constituent of the extracellular matrix.[9-11]

Measurement of plasma levels of β-thromboglobulin and platelet factor 4, two secreted platelet proteins, allows the detection of in vivo platelet activation and the distinction with artifactual in vitro release.[12, 13]

Elevated plasma levels of fibrinopeptide A, a cleavage product of the α-chain of fibrinogen and of thrombin–antithrombin III complexes, are indices of thrombin and fibrin generation.[14]

In the current study, with increased plasma fibronectin used as an early predictor of the development of gestational hypertension, we investigated the time course of changes in plasma levels of the above-mentioned proteins, in an attempt to define the sequence of pathologic events leading to the clinical picture.

Material and methods

A total of 105 apparently healthy normotensive primigravid women, seen at the outpatient clinic of the Department of Obstetrics between September 1988 and June 1989, were prospectively included in the study after giving informed consent. The nature of the study was explained in detail and none of the subjects who were requested to participate declined.

Blood pressure was measured in the sitting position. Diastolic pressure was defined as the pressure at which a Korotkoff phase V was obtained. Blood pressure was

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6/1/32023

Table I. Blood pressure, parameters of vascular damage, platelet activation, and coagulation in 14 normotensive primigravid women and in 10 primigravid women in whom preeclampsia developed

Parameter	*Third-trimester control group*	*Weeks before onset of clinical symptoms*			
		−4	*−2*	*−1*	*0*
Systolic pressure (mm Hg)	120.6 ± 7.5	127 ± 8 ($p < 0.05$)	126 ± 9.8 (NS)	128 ± 9.7 ($p < 0.05$)	151.5 ± 14 ($p < 0.0001$)
Diastolic pressure (mm Hg)	72 ± 5.8	73 ± 4.6 (NS)	77.1 ± 4 (NS)	80 ± 3.8 ($p < 0.005$)	99 ± 7 ($p < 0.0001$)
Fibronectin (μg/ml)	332 ± 68	606 ± 129 ($p < 0.0001$)	647 ± 136 ($p < 0.0001$)	674 ± 140 ($p < 0.0001$)	790 ± 200 ($p < 0.0001$)
Laminin (IU/ml)	2.3 ± 0.5	3 ± 1.0 ($p < 0.05$)	3.2 ± 0.6 ($p < 0.005$)	3.2 ± 0.6 ($p < 0.005$)	3.3 ± 0.6 ($p < 0.005$)
Preprocollagen (IU/ml)	1.2 ± 0.4	1.3 ± 0.5 (NS)	1.6 ± 0.6 (NS)	1.9 ± 0.5 ($p < 0.01$)	2.3 ± 0.8 ($p < 0.005$)
β-Thromboglobulin (IU/ml)	19.6 ± 10	56.1 ± 29.3 ($p < 0.005$)	48.2 ± 27.7 ($p < 0.005$)	42.9 ± 26 ($p < 0.005$)	50.5 ± 28.5 ($p < 0.0001$)
Platelet factor IV (IU/ml)	4 ± 2.5	22.7 ± 16.6 ($p < 0.005$)	14 ± 12 ($p < 0.005$)	8.5 ± 6.1 ($p < 0.05$)	13.7 ± 12.2 ($p < 0.005$)
Thrombin–antithrombin complex (IU/ml)	5.9 ± 2.2	7.6 ± 2.6 (NS)	9.8 ± 3.3 ($p < 0.005$)	13.8 ± 9.7 ($p < 0.0001$)	12 ± 4.2 ($p < 0.005$)
Fibrinopeptide A (ng/ml)	9 ± 7.9	18 ± 28 (NS)	20.3 ± 25 (NS)	22.3 ± 21 (NS)	34 ± 27 (NS)
Platelets (10^9/L)	249 ± 68	282.5 ± 66.6 (NS)	255 ± 74 (NS)	257 ± 47 (NS)	239 ± 92 (NS)
Hematocrit (ratio)	35 ± 3.2	32.6 ± 3.3 (NS)	34.6 ± 3.9 (NS)	36.7 ± 2.9 (NS)	38 ± 1.8 ($p < 0.01$)

Data represent mean ± SD and p value. *NS*, Not significant.

measured before 20 weeks of pregnancy, monthly between 20 and 32 weeks, every 2 weeks between 32 and 36 weeks, and then weekly until delivery. Gestational hypertension was defined as a diastolic blood pressure of >90 mm Hg on at least two occasions during the second half of pregnancy in a previously normotensive woman. Preeclampsia was defined as increased diastolic blood pressure and a proteinuria of 0.3 gm/day (International Society for the Study of Hypertension in Pregnancy).[15]

Management of the subjects was determined on the basis of clinical signs and was not altered on the basis of the results of blood analyses. Blood samples for fibronectin were taken three times during pregnancy at 28, 32, and 36 weeks. In 10 patients, where the level of fibronectin was >450 μg/ml (mean ± 2 SD), additional blood samples for laminin, preprocollagen III, β-thromboglobulin, platelet factor 4, thrombin–antithrombin III complexes, and fibrinopeptide A were taken 2 weeks later and then weekly until development of clinical symptoms of gestational hypertension. Fourteen patients, selected at random, without any rise of plasma fibronectin level and who remained normotensive during pregnancy were taken as controls. The subjects were asked to refrain from aspirin intake during the study.

All blood samples were drawn from fasting subjects without stasis before 10 AM, by venipuncture, after a rest period of at least 10 minutes. For β-thromboglobulin and platelet factor 4, blood was mixed 9:1 in special tubes (Diatubes-H, Stago, France) containing a mixture of theophylline, adenosine, and dipyridamole. Samples were immediately mixed and kept on ice until centrifugation for 30 minutes, at 2500g at 4° C. The supernatant was transferred in plastic tubes with a plastic pipette and stored at −20° C. All tests were performed within 30 days after sampling with enzyme-linked immunosorbent assay (ELISA, Stago). For fibronectin, laminin, preprocollagen III, and thrombin–antithrombin III complexes, the blood was anticoagulated with buffered citrate (nine parts of blood were mixed with one part of sodium citrate, 38 mg/ml) and centrifuged for 10 minutes at 2500g at 4° C.

Fibronectin was measured by the Mancini immunodiffusion technique with commercial plates from Behringwerke (Marburg Lahn, West Germany). The level of plasma fibronectin throughout normal pregnancy was 330 μg/ml or 100%, the cutoff point is taken at 450 μg/ml or 140% (mean ± 2 SD).[8] Laminin and preprocollagen III were determined by commercial radioimmunoassay kits and thrombin–antithrombin III complexes with an enzyme-linked immunosorbent assay test also purchased from Behringwerke. If not analyzed immediately, the samples were stored at −20° C. For measurement of fibrinopeptide A an enzyme-linked immunsorbent assay test from Stago was used. Blood for fibrinopeptide A was quickly collected in a special anticoagulation solution containing citrate, heparin, and a protein-specific inhibitor, rapidly centrifuged for 10 minutes at 2500g and treated with ben-

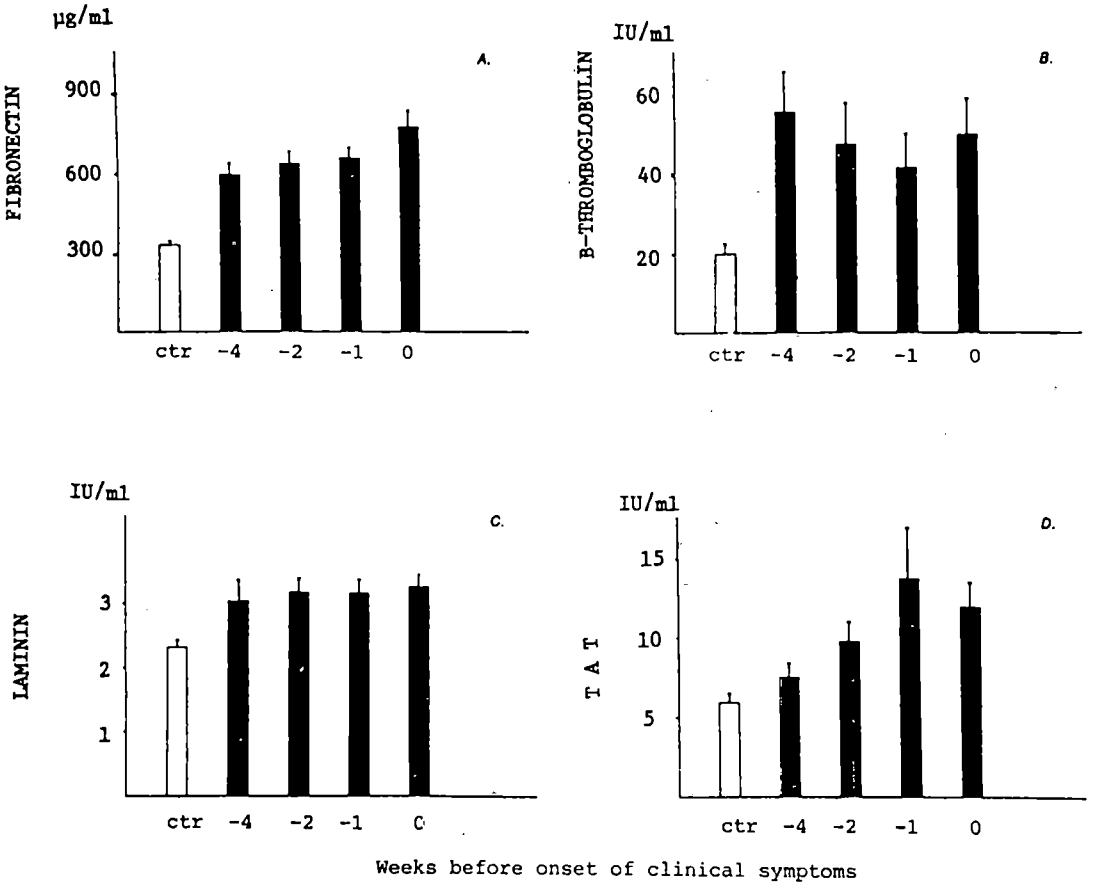


Fig. 1. Plasma levels of fibronectin **(A)**, β-thromboglobulin **(B)**, laminin **(C)**, and thrombin–antithrombin III complex *(TAT)* **(D)** during 30 to 40 weeks of pregnancy in 14 normotensive women (control group) and in 10 women in whom gestational hypertension developed at weeks −4, −2, and −1 before clinical symptoms and at diagnosis *(0)* of gestational hypertension. Results in columns represent means; *bars*, SEM.

tonite (1 ml plasma with 0.5 ml bentonite centrifuged at 1400*g* for 10 minutes and stored −20° C). The Mann-Whitney rank-sum test was used to determine the differences between the groups. Correlation between different parameters were measured with the Spearman test.

Results

Ten of the 105 initially normotensive primigravid women had elevated fibronectin plasma levels, >450 µg/ml, which is two standard deviations above the mean. Seven of the 10 patients had a significant fibronectin rise later than 32 weeks. Of those 10 patients, eight had severe preeclampsia with proteinuria, two had gestational hypertension, and none had eclampsia or severe coagulation defects. The 95 with normal fibronectin levels remained normotensive.

Table I summarizes the results of blood pressure, plasma levels of fibronectin, laminin, and preprocollagen III, plasma levels of β-thromboglobulin and platelet factor 4, plasma levels of fibrinopeptide A, thrombin–antithrombin III complexes, and platelet count and hematocrit at regular intervals in the third trimester of the women in whom gestational hypertension developed, compared with the normotensive controls. At the moment of clinical disease (time = 0) all the studied parameters of endothelial damage, platelet activation, and coagulation were significantly different compared with the control group. The hematocrit was also higher, but we did not find a significant difference in platelet count. When diagnostic category was subdivided, levels of fibronectin (565 ± 7 vs 848 ± 182 µg/ml) and laminin (2.9 ± 0.18 vs 3.39 ± 0.68 IU/ml) were higher in the preeclampsia with proteinuria ($n = 8$) versus the nonproteinuria group ($n = 2$).

Four weeks before clinical symptoms (compared with the control group), significant increases in plasma levels of fibronectin ($p < 0.0001$) and laminin ($p < 0.05$), which are parameters of early vascular damage, and β-thromboglobulin and platelet factor 4 ($p < 0.005$), which are indices of platelet activation, were found (Fig. 1). Of the parameters of coagulation, thrombin–

antithrombin III complexes were significantly raised 2 weeks later ($p < 0.005$), whereas a steady but not significant increase was found for fibrinopeptide A. Preprocollagen III was significantly increased only 1 week before symptoms ($p < 0.01$). In the group who finally became hypertensive, we found a significant correlation ($p < 0.01$) between fibronectin and diastolic blood pressure ($r = 0.994$), laminin ($r = 0.974$), fibrinopeptide A ($r = 0.994$), and preprocollagen III ($r = 0.970$). No such correlations were found in the control group.

Comment

Gestational hypertension is a rather common complication of pregnancy, peculiar to humans and affecting both maternal and fetal health. Its cause and pathogenesis remain enigmatic and controversial. In established preeclampsia, platelet activation and chronic coagulopathy, which enhance the gravity of the symptoms, have been well characterized.[16]

Recently, however, the role of endothelial cell damage in the development of the clinical and biochemical features of the disease has been stressed.[17] Although the mechanism is not fully understood, it would be interesting to find early ways of measuring this damage. A predictive marker would indeed be of clinical value particularly for the timely start of a prophylactic treatment and for the evaluation of the progress of the disorder.

In the current study we prospectively analyzed women in whom hypertension ultimately developed. They showed an early rise, at least 4 weeks before the onset of clinical symptoms, of fibronectin, laminin, and later preprocollagen type III. An increase in plasma fibronectin levels correlates well with the increase of other basement membrane proteins, such as laminin and preprocollagen III, indicating a continuous process. These changes are compatible with endothelial cell damage, causing exposure of the basement membrane and the subendothelium. In response to this injury platelets are activated and adhere to subendothelial structures, such as collagen III and laminin. The simultaneous significant rise of β-thromboglobulin and, to a lesser degree, of platelet factor 4 found in the study indicates platelet activation and release of the contents of the α-granules. The release of the vasoconstrictor thromboxane A_2 by the damaged endothelial cells, together with the release of vasoactive substances such as serotonin and others, by the α-granules of platelets could be responsible for the onset of hypertension. Higher levels of urinary metabolites of thromboxane A_2 have also been described in a preclinical stage.[18] These activated platelets may cause vasoconstriction and activate coagulation. Finally, signs of fibrin formation occurred somewhat later; there was a significant rise of the thrombin–antithrombin III complexes from week −2. There was a steady but nonsignificant rise of fibrinopeptide A, however, with a significant correlation of fibrinopeptide A with the fibronectin level. This discrepancy may be explained partly by the high standard deviation. The absence of correlation between thrombin–antithrombin III complexes and fibronectin and the nonsignificant rise of fibrinopeptide A might be the result of the low degree of severity of the disease in most patients, as other authors have clearly found a positive correlation between plasma antithrombin III activity and the degree of severity of preeclampsia and eclampsia.[19] This secondary activation of the coagulation, combined with a decrease of fibrinolysis and impaired fibrin clearance, will obviously interfere with the microcirculation of the placenta, liver, central nervous system, and kidneys.

Our study indicates that indices of endothelial damage and platelet activation antedate parameters of coagulation and the clinical onset of symptoms in gestational hypertension. They offer possibilities of early diagnosis and assessment of therapeutic approaches aimed at limiting vascular damage and platelet activation.

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Human monoclonal antibody recognizing an antigen associated with ovarian and other adenocarcinomas

Lloyd H. Smith, MD, PhD,[a]* Amy Yin, MD,[b] Michelle S. Glasky, PhD,[b] Nancy Tyler, PhD,[c] Mariana Robles, MS,[c] Chris A. Foster, BS,[a] Marcia Bieber, MD,[b] and Nelson N.H. Teng, MD, PhD[b]

Davis and Stanford, California

MS2B6, a human monoclonal antibody derived from a patient with advanced ovarian cancer, has been used to study the distribution and characteristics of its target antigen. The MS2B6 antigen was detected by immunoperoxidase studies in 41 of 41 epithelial ovarian cancers and in the majority of nonovarian adenocarcinomas. Among normal tissues the MS2B6 antigen was found in the adult epithelia of the fallopian tube, endometrium, endocervix, colon, bronchus, breast, sweat duct, and large renal ducts. No detectable antigen was found in peritoneal epithelia, tissue stromal cells, spleen, thymus, or blood-borne cells. Immunoblotting analysis showed that the MS2B6 epitope resides on polypeptides of 38, 44, and 60 kd. The cellular location of the MS2B6 antigen was studied with immunoperoxidase and immunofluorescent staining and immunoelectronmicroscopy of ovarian cancer ascites tumor cells. The results suggest that in ascites tumor cells the MS2B6 antigen is located in a layer of the peripheral cytoplasm beginning just below the cell membrane. MS2B6 may be useful as an imaging or therapeutic agent. (AM J OBSTET GYNECOL 1992;166:634-45.)

Key words: Human monoclonal antibodies, ovarian cancer, immunoperoxidase

The advent of mouse monoclonal antibody (MAb) technology has provided a long list of potentially useful reagents for diagnosis, treatment, and management of clinical problems in obstetrics and gynecology.[1] For example, CA 125 is an ovarian cancer–associated antigen detected by mouse MAb OC 125, which has been extensively studied.[2] Although the usefulness of OC 125 in screening for asymptomatic ovarian cancer remains uncertain,[3] it has an established role in several areas of gynecology, including triage of patients with pelvic masses[4] and management of most patients with nonmucinous epithelial malignancies of the ovary.[5] OC 125 has emerged as one of the best examples of the potential utility of monoclonal antibody reagents in clinical medicine. However, limitations are recognized; when mouse MAbs are administered in vivo to patients, the result is often a human antimouse antibody (HAMA) response. The HAMA response occurs more frequently after multiple administrations of mouse MAbs[6]; it may occur in spite of immunosuppressive therapy[7] and may cause adverse clinical sequelae or an abrogation of the intended diagnostic or therapeutic effect.[8,9] In the case of mouse MAb OC 125, a HAMA response was detected in three of seven patients given intravenous injection of radiolabeled OC 125 for imaging and six of eight patients given radiolabeled OC 125 for therapy.[10] Furthermore, patients once exposed to mouse MAb may be ineligible for subsequent clinical trials with other mouse MAbs because the presence of HAMA can affect mouse MAb metabolism. The presence of HAMA can also interfere with certain laboratory tests based on mouse MAb technology.

Because of the HAMA phenomenon interest has increased in the development of genetically engineered chimeric human-mouse MAbs[11] and the generation of human MAbs to cancer-associated antigens.[12] The study of human MAbs directed toward antigens associated with cancer cells may not only provide potentially useful MAbs reagents that are presumably less immunogenic for humans than mouse MAbs, they may also allow an improved understanding of the human immune response to malignancy. In fact, the humoral immune response to cancer-associated antigens can be directly studied in detail with human MAbs and can only be indirectly approached with cancer-associated antigens detected by mouse MAbs.

We have previously described an efficient method of screening human MAbs produced by Epstein-Barr vi-

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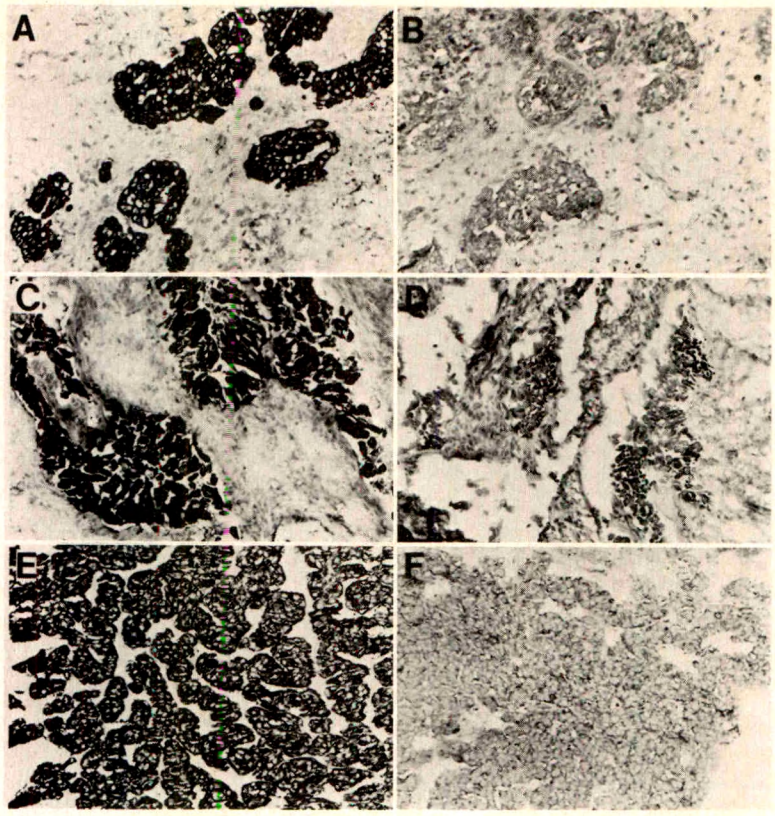


Fig. 1. Distribution of MS2B6 target antigen in ovarian cancer tissues; immunoperoxidase staining as described in text. **A,** Metastatic, poorly differentiated serous papillary ovarian carcinoma (Biotin-labeled MS2B6 stain; ×200); **B,** Same tissue as **A** (Biotin-labeled negative control IgM stain); **C,** Metastatic, poorly differentiated mucinous ovarian carcinoma (Biotin-labeled MS2B6 stain; ×200); **D,** Same tissue as **C** (Biotin-labeled negative control IgM stain); **E,** Moderately differentiated endometrioid ovarian carcinoma (Biotin-labeled MS2B6 stain; ×200); **F,** Same tissue as **E** (Biotin-labeled negative control IgM stain).

rus–transformed patient lymphocytes for binding to purified ovarian cancer ascites tumor cells.[13] MS2B6 is a human monoclonal immunoglobulin M (IgM) (μ, λ) derived from lymphocytes from a patient with serous papillary ovarian carcinoma. Here we describe the preliminary characterization of the MS2B6 antigen, including its tissue distribution, molecular characteristics, and cellular location.

Material and methods

Purification and labeling of MS2B6 IgM. Derivation of human hybridoma MS2B6 has been described previously.[13] In brief, splenocytes from a patient with stage III ovarian cancer were obtained from splenic tissue removed at tumor debulking. These lymphocytes were Epstein-Barr virus transformed and grown at 1000 cells per well; after 3 weeks supernatants were screened for binding to ovarian cancer ascites tumor cells. Lymphocytes from a positive well were fused with heteromyeloma SHM-D33, and hybrid cells were selected. A hybridoma line that secreted the MS2B6 IgM was identified. The cell line was easily adapted for growth in serum-free medium (Iscoves with 1% Nutridoma-NS, Boehringer-Mannheim, Indianapolis) and continued to produce IgM at a rate of 32 μg per 10^6 cells per day. Supernatant fluids containing approximately 60 μg/ml IgM were concentrated 100-fold by ultrafiltration with a membrane with 300,000 d molecular weight cut-off

Table I. MS2B6 immunoperoxidase studies: Epithelial ovarian cancer

Subtype	*Borderline*	*Tumor grade*			*Total*
		I	*II*	*III*	
Papillary serous	2/2*	NT	10/10	18/18	30/30
Mucinous	1/1	1/1	NT	2/2	4/4
Endometrioid	NT	1/1	3/3	2/2	6/6
Clear cell	NT	NT	NT	1/1	1/1
TOTAL					41/41

NT, None tested.

*Number of patients with positive test results divided by number of patients tested.

Table II. MS2B6 Immunoperoxidase studies: Other cancers

Tumor type	*Origin*	*MS2B6 Staining*
Squamous cell carcinoma	Cervix	0/4*
Squamous cell carcinoma	Vulva	0/3
Squamous cell carcinoma	Ovarian teratoma	0/1
Adenocarcinoma	Cervix	1/1
Adenocarcinoma	Endometrium	5/10
Adenocarcinoma	Breast	2/2
Adenocarcinoma	Lung	1/1
Adenocarcinoma	Colon	5/5
Adenocarcinoma	Pancreas	1/1
Adenocarcinoma	Ovarian Krukenberg tumor	1/1
Undifferentiated carcinoma	Pelvis (unknown primary)	1/3
Mesothelioma	Peritoneum	1/1
Transitional cell carcinoma	Kidney	1/1
Leiomyosarcoma	Uterus	0/1
Malignant mixed müllerian tumor	Uterus	0/2
Malignant mixed müllerian tumor	Ovary	0/1
Adenocarcinoma	Uterus	0/1
Immature teratoma	Ovary	1/1
Endodermal sinus tumor	Ovary	0/1
Dysgerminoma	Ovary	0/1
Melanoma	Peritoneal cavity	0/2
Lymphoma	Omentum	0/1

*Number of patients with positive test results divided by number of patients tested.

(Amicon, Beverly, Mass.). The MS2B6 IgM was purified by high-pressure ion exchange chromatography with a liquid chromatograph (model 1084B, Hewlett Packard, Palo Alto, Calif.) and a 0.75 × 7.5 cm TSK-DEAE-5PW column (Bio Rad, Hercules, Calif.). In brief, buffer A consisted of 0.02 mol/L Tris, 0.1 mol/L sodium chloride, pH 8.5; buffer B consisted of 0.02 mol/L Tris, 0.6 mol/L sodium chloride, pH 7.0. Samples were applied to the column, which had been equilibrated with 10% buffer B in buffer A; the IgM was eluted by changing to 25% buffer B. The column was washed with 100% buffer B and finally 10% buffer B for regeneration. The eluted IgM was concentrated and stored frozen in liquid nitrogen. The purity of the final product was approximately 95% IgM by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Biotin labeling was by the method of Haspel et al.[14] with modifications. High-pressure ion exchange chromatography–purified MS2B6 IgM or control IgM (purified human serum IgM, Cappel, Durham, N.C.) at 1 mg/ml was dialyzed versus 0.01 mol/L potassium phosphate and 0.15 mol/L potassium chloride, pH 7.8. Biotin: *N*-hydroxysuccinimide (Sigma Chemical Co., St. Louis) dissolved at 2 mg/ml in dimethylsulfoxide was added to give a molar ratio of biotin to IgM of 30:1 (with a molecular weight for monomer IgM of 180,000 d). After 15 minutes at room temperature with agitation, 0.1 vol 1 mol/L ammonium chloride was added and the solution dialyzed versus phosphate-buffered saline solution with 0.02% sodium azide.

Immunoperoxidase studies. Procurement of human tissues had the approval of the Human Subjects Committee at Stanford University and University of California–Davis School of Medicine and was obtained after surgery or autopsy through the Departments of Surgical Pathology. Pathology reports on each malignant tissue were reviewed. The fresh tissues were cut into small pieces, wrapped in foil, and stored in liquid nitrogen until immediately before transfer to embedding medium. Six-micron cryostat sections were air dried and stored at 4° C, then fixed immediately before use with PLP fixative[15] (0.5% paraformaldehyde, 0.075 mol/L L-lysine, and 0.01 mol/L sodium periodate) for 10 minutes at 4° C followed by washing in phosphate-buffered saline solution. Some tissues required blocking of endogenous biotin as described elsewhere.[16] Suppression of endogenous peroxidase activity was performed as described elsewhere.[17] Immunoperoxidase staining with biotin-labeled MS2B6 IgM or control IgM was performed in the standard manner,[18] in brief as follows: The sections were sequentially incubated (with phosphate-buffered saline solution washing between incubations) with 10% fetal calf serum to block, biotin-labeled antibody at 25 μg/ml, streptavidin-HRP (Sigma) at 0.5 μg/ml, then diaminobenzidine (DAB, Sigma) at 1 mg/ml in 0.1 mol/L Tris pH 7.2 with 0.03% hydrogen peroxide. After 15 minutes slides were washed with water, counterstained with hematoxylin, and covered with coverslips. Microscopically, positive staining by biotin-labeled MS2B6 IgM was visualized as deposition of brown stain on cells not stained by biotin-labeled control IgM.

Electrophoresis and immunoblotting. SDS-PAGE was performed with the discontinuous buffer system of Laemmli,[19] with 4% stacking and 10% running gels. Samples consisted of purified ovarian cancer ascites tumor cells or ovarian cancer cell line 2774. Preparation of the ascites tumor cells is described below. The ovarian cancer cell line 2774 was grown to confluence in Dulbecco's modified Eagle's medium with 10% fetal calf serum, scraped off the flasks, and washed in phosphate-buffered saline solution. Sodium dodecyl sulfate sample buffer containing 2-mercaptoethanol was added (20 μl/10^6 cells) and the samples heated to 100° C for 3 minutes. Human IgM (2 μg) was run in control lanes to confirm transfer after immunoblotting.

Blotting onto nitrocellulose paper was as described by Towbin et al.[20] The blots were blocked by incubation in 4% nonfat milk in phosphate-buffered saline solution for 1 hour at room temperature. Indirect immu-

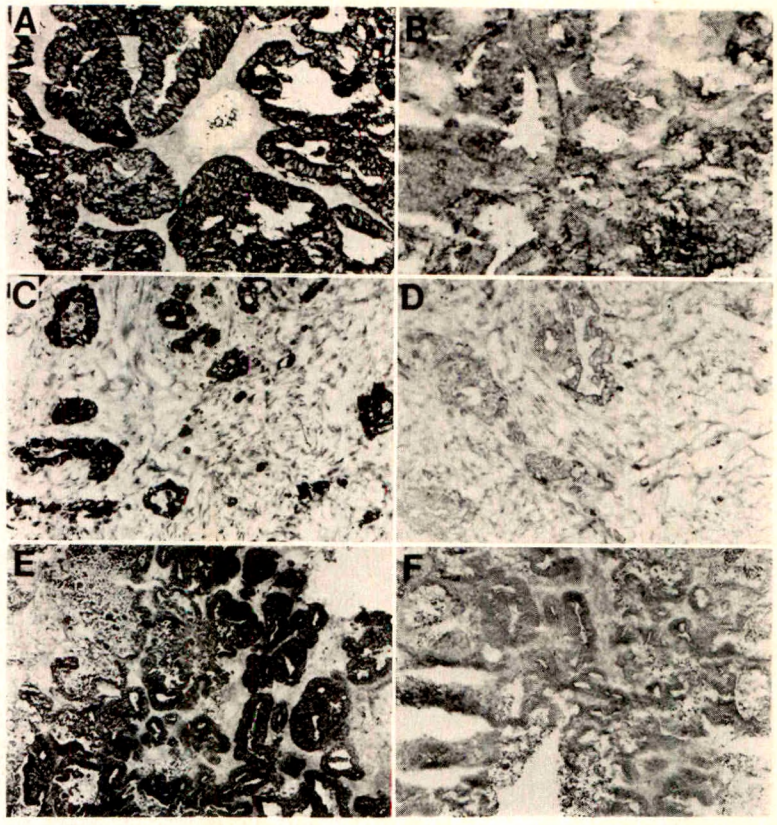


Fig. 2. Distribution of MS2B6 target antigen in nonovarian adenocarcinoma tissues. **A,** Moderately differentiated endometrial carcinoma (Biotin-labeled MS2B6 stain; ×200); **B,** Same tissue as **A** (Biotin-labeled negative control IgM stain); **C,** Metastatic pancreatic carcinoma (Biotin-labeled MS2B6 stain; ×200); **D,** Same tissue as **C** (Biotin-labeled negative control IgM stain); **E,** Colon carcinoma (Biotin-labeled MS2B6 stain; ×200); **F,** Same tissue as **E** (Biotin-labeled negative control IgM stain).

nostaining was performed by the alkaline phosphatase method[21] as modified by Sidberry et al.[22] Unlabeled MS2B6 or control IgM (human serum IgM, Cappel) were incubated with the blot at 5 to 10 μg/ml for 4 hours followed by PBS washing. Next the blots were incubated with alkaline phosphatase–labeled goat antihuman IgM (1:200, Sigma) for 2 hours, washed in phosphate-buffered saline solution, then placed in 50 mmol/L Tris, pH 8.3. Freshly prepared chromagen solution (Naphthol AS-MX phosphate 1 mg/ml, Fast Red TR salt 2 mg/ml, both from Sigma, in 50 mmol/L Tris, pH 8.3) was added and incubation continued until color development was achieved. The blots were then washed with water and air dried.

Immunolocalization experiments. Three ovarian cancer ascites tumor cell preparations purified by Percoll buoyant centrifugation[13] were used for the immunoperoxidase studies. Tumor cells were either fixed in the PLP fixative (as described above) or in methanol-acetone (1:1) for 10 minutes at 4° C. Fixed cells were reacted with biotin-labeled MS2B6 or biotin-labeled control IgM, washed, reacted with streptavidin-peroxidase, and developed with DAB as described in the immunoperoxidase tissue studies. After washing, the cells were fixed with 2% glutaraldehyde in phosphate-buffered saline solution for 30 minutes at room temperature to stabilize the DAB-stained cells. The cells were subsequently stained with 2% osmium tetroxide for 30 minutes, dehydrated in alcohol, equilibrated with propylene oxide, infiltrated with epoxy (Polybed 812, Polysciences, Warrington, Pa.), and polymerized at 60° C. Two-micron sections were obtained for microscopy without further counterstaining. For immunoelectronmicroscopy, the methanol-acetone–fixed, epoxy-embedded cell blocks were sectioned at 80 to 100 nm with an ultramicrotome (Reichert, model OMU3, American Optical, Southbridge, Mass.) mounted on 200 mesh copper/palladium grids. To maximize the contrast of the DAB deposits, these sections were not counterstained further. The grids were viewed with a Philips electron microscope. Photographs were printed from negatives to give identical contrast levels.

Immunofluorescence studies were performed with ovarian cancer ascites tumor cell preparations that had

Table III. MS2B6 Immunoperoxidase studies: Normal human tissues

Tissue type	*MS2B6 Staining*	*Staining characteristics*
Ovary	0/5*	
Fallopian tube	4/4	Epithelium only
Myometrium	0/4	
Endometrium	3/4	Epithelium only
Endocervix	2/2	Epithelium only
Vagina	0/1	
Peritoneum	0/4	
Bladder	1/1	Epithelium only
Kidney	3/4	Large tubular epithelium only
Prostate	2/2	Epithelium only
Salivary gland	1/1	Epithelium only
Esophagus	0/1	
Stomach	0/1	
Small bowel	1/1	Weak staining, epithelium only
Gall bladder	0/1	
Colon	4/4	Epithelium only
Liver	1/4	One specimen, weak staining
Lung	3/3	Bronchiolar epithelium only
Breast	3/3	Ductal epithelium
Skin	1/1	Sweat duct epithelium only
Spleen	0/2	
Thymus	0/1	
Brain	0/2	
Thyroid	1/2	One specimen, very weak patchy staining
Placenta, mid-trimester	1/2	Syncytiotrophoblast epithelium
Placenta, term	1/1	Syncytiotrophoblast epithelium, patchy staining
Fetal intestine	1/1	
Fetal liver	6/7	
Fetal skin	0/2	
Fetal kidney	1/2	One specimen, weak staining
Fetal bladder	1/1	

*Number of patients with positive test results divided by number of patients tested.

been cryopreserved. After the cells were thawed and washed, they were reacted with biotin-labeled MS2B6 or biotin-labeled control IgM. Subsequently the cells were washed and reacted with streptavidin-fluorescein isothiocyanate (Sigma) in phosphate-buffered saline solution and washed and viewed by ultraviolet microscopy. Where indicated, propidium iodide (Sigma) 1 μg/ml was included in the final wash buffer.

CA 125 immunoassay. An enzyme-linked immunoassay for ovarian cancer–associated antigen CA 125 was performed according to the insert in the test kit (Abbott Laboratories, Abbott Park, Ill.). To 100 μl of CA 125 standard (168 U/ml) were added aliquots of OC 125 (gift of R. Knapp) or MS2B6. After subsequent incubation with OC 125–beads and OC 125–peroxidase conjugate, the beads were washed and developed and optical density measurements were obtained (495 nm).

Results

Tissue distribution of MS2B6 antigen. To maximally preserve native antigen structures for immunoperoxidase studies, frozen sections were used. Fixation of the sections with PLP allowed adequate tissue preservation and antigen staining. Forty-one different ovarian epithelial cancer tissue samples were stained for biotin-labeled MS2B6 binding. As shown in Table I, 41 of 41 specimens stained positively, including 30 serous papillary, 4 mucinous, 6 endometrioid, and 1 clear-cell tumor. The single example of a borderline mucinous tumor from a patient with pseudomyxoma peritonei demonstrated marked heterogeneity of expression of MS2B6 antigen with weak, patchy staining. Heterogeneous expression of the antigen was absent or minimal in the other tissues examined, which showed remarkable uniformity in staining. Fig. 1 demonstrates the pattern of staining for ovarian carcinoma tissues. Among the serous carcinomas, positive staining was independent of tumor grade.

Forty-five different nonovarian or nonepithelial ovarian malignant tissues were examined for MS2B6 antigen staining (Table II). Eight examples of squamous cell carcinoma, 5 sarcomas, and 2 of 3 ovarian germ cell cancers were negative for MS2B6 antigen expression. One ovarian immature teratoma did have positive staining, but only in areas of mature, benign epithelium. The majority of 20 nonovarian adenocarcinomas, including 5 of 10 endometrial adenocarcinomas and five of five colon adenocarcinomas stained positively for MS2B6 antigen (Fig. 2). In addition, one of three undifferentiated carcinomas (unknown primary), a mesothelioma (peritoneum), and a transitional cell carcinoma (kidney) stained positively. Examples of malignant melanoma and lymphoma stained negatively.

Seventy-three different normal adult- and fetal-tissue specimens were examined for MS2B6 antigen staining (Table III). Positive staining was found among certain normal nonsquamous epithelia, including fallopian tube, endocervix, endometrium, bronchus, colon, mammary ducts, large renal ducts, salivary glands, sweat ducts, and bladder. Weak, patchy staining among normal tissues was noted in single examples of liver, thyroid, and small bowel epithelium. The strongest and most uniform staining was of fallopian tube, endometrial, and bronchial epithelia (Fig. 3).

Several tissues, including kidney, liver, and colon, demonstrated significant staining of epithelia when the biotin-labeled negative control IgM preparation was used to stain the tissues. In the case of liver the vast majority of this background binding was removed by blocking for endogenous biotin activity. Even after the blocking of endogenous biotin, several specimens of colon epithelium demonstrated significant binding of negative control biotin-labeled IgM, although binding of biotin-labeled MS2B6 was always more intense. Most of this background control IgM staining of colon epithelium could be removed by blocking for endogenous

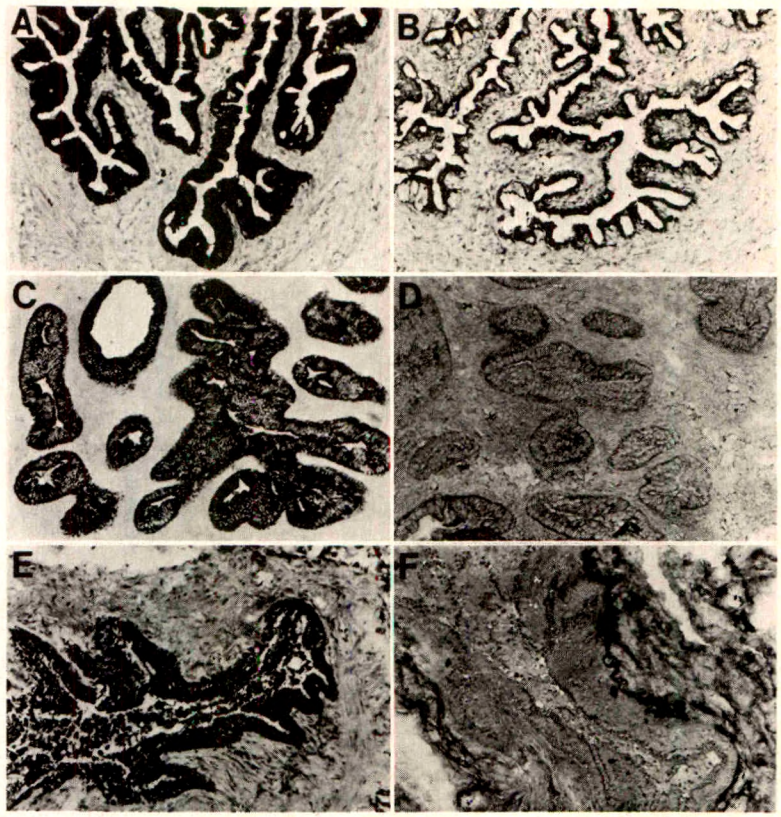


Fig. 3. Distribution of MS2B6 target antigen in normal adult epithelia. **A,** Fallopian tube (Biotin-labeled MS2B6 stain; ×200); **B,** Same tissue as **A** (Biotin-labeled negative control IgM stain); **C,** Endometrium (Biotin-labeled MS2B6 stain; ×200); **D,** Same tissue as **C** (Biotin-labeled negative control IgM stain); **E,** Bronchus (Biotin-labeled MS2B6 stain; ×200); **F,** Same tissue as **E** (Biotin-labeled negative control IgM stain).

peroxidase. A similar phenomenon was observed for several specimens of kidney epithelium. Here, MS2B6 staining was weak and generally confined to the epithelium of large ducts, with glomeruli being totally negative. Control IgM staining of kidney specimens resulted in a similar but even less intense pattern, even after blocking the endogenous biotin.

The absence of the MS2B6 antigen from peritoneal and ovarian epithelia deserves note. In addition, the antigen was not detected among epidermoid epithelia except in the sweat gland ducts in the skin. Among fetal tissues the antigen was intensely expressed in examples of syncytiotrophoblast epithelium and in fetal intestinal, hepatic, and renal epithelia. Six specimens of benign lesions were negative for MS2B6 antigen, including 2 benign ovarian cysts, 2 uterine leiomyomas, 1 ovarian fibroma, and 1 hydatidiform mole. Two of three cases of endometriosis stained positively.

In addition to the absence of MS2B6 staining of normal spleen and thymus, other lymphoid collections such as those located in intestinal submucosa stained negatively. Among all tissues examined, intravascular elements always stained negatively for MS2B6 staining. To independently test for MS2B6 binding to elements of the blood (e.g., red blood cells, lymphocytes, granulocytes, platelets), further tests were performed. MS2B6 at 50 μg/ml was tested by the Stanford Transfusion Service with standard hemaagglutination techniques. MS2B6 failed to react with a panel of red blood cells expressing the following antigens: blood group antigens A and B; Rh antigens D, C, c, E, and e; Kell antigens K, k, Kp^b, and Js^b; Duffy antigens Fy^a and Fy^b; Kidd antigens Jk^a and Jk^b; Lewis antigens Le^a and Le^b; P antigen; MN antigens M, N, S, and s; Lutheran antigens Lu^a and Lu^b; and $X^a{}_g$ antigen. MS2B6 (at 50 μg/ml) was also tested for granulocyte agglutination and for cytotoxicity to granulocytes, lymphocytes, and platelets based on a carboxyfluorescein diacetate uptake technique.[23] Four different granulocyte specimens were negative for MS2B6-induced aggregation or cytotoxicity. Four different lymphocyte specimens and six different platelet preparations showed no MS2B6-induced cytotoxicity.

Western blot analysis. Western blot analysis of whole cell extracts of both purified ascites cells and ovarian

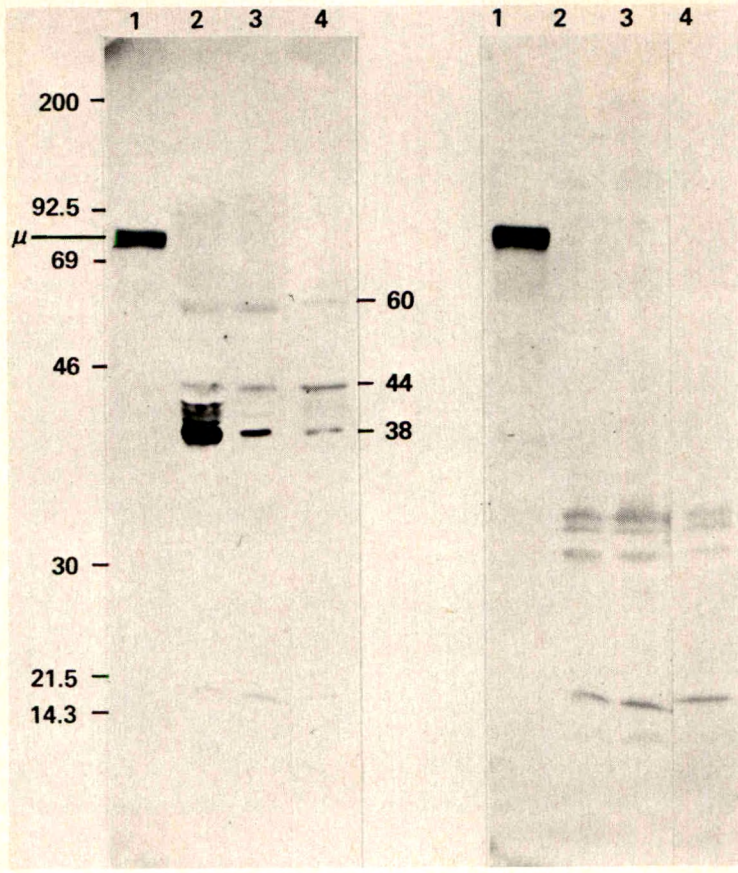


Fig. 4. Immunoblot analysis of MS2B6-reactive material. *Left,* Immunoblot. *Lane 1:* human IgM, 2 μg; *lane 2:* ascites tumor cells (patient A.B.), whole cell extract; *lane 3:* ascites tumor cells (patient L.L.), whole cell extract; *lane 4:* ovarian cancer cell line 2774, whole cell extract (MS2B6 stained). *Right,* Immunoblot. Lanes same as in *left.* (Negative control IgM stain).

cancer cell line 2774 consistently revealed several MS2B6-staining bands not observed in blots stained with negative control IgM (human serum IgM) (Fig. 4). The major components consisted of bands of approximately 38 and 44 kd. Although some heterogeneity was apparent, the 38 kd species was the prominent band among ascites tumor cell preparations. In some preparations a 60 kd band was present, but this band was less intense than the 38 or 44 kd bands and was seen inconsistently. None of these bands appeared after staining identical blots for control IgM binding.

Cellular localization of MS2B6 antigen. Ovarian cancer ascites tumor cells were chosen for these studies to achieve improved resolution over that obtained in the tissue frozen-section studies. Previous immunofiltration studies with cryopreserved ascites tumor cells suggested the possibility that MS2B6 was reacting to an antigen on the tumor cell surface. Two methods of fixation were chosen. The PLP fixative was chosen to preserve the integrity of the cell membranes, and the methanol-acetone fixative was used to permeabilize the cells, allowing cell entry of the MS2B6 IgM molecule. MS2B6-stained tumor cells had a dark ring around the cell periphery (Fig. 5). The pattern suggested the possibility of surface staining, but many more cells stained after methanol-acetone fixation (91%) than after PLP fixation (31%), a finding more consistent with an intracellular location of the MS2B6 antigen.

Immunofluorescence studies on unfixed ascites tumor cells were performed to assess the possibility of surface staining. Fig. 6 shows that MS2B6-stained cells had fluorescence at the cell periphery. The control IgM–stained cells had no fluorescence (not shown). However, the number of MS2B6-stained cells with such peripheral ring fluorescence was proportional to the number of nonviable cells (as judged by trypan blue exclusion) present in the three ascites specimens examined. When these experiments (including a propidium iodide counterstain to reveal nonviable cells) were repeated, almost all cells with MS2B6 ring fluorescence also had red propidium iodide nuclear staining (not shown). Ascites tumors cells that were judged viable by the lack of propidium iodide nuclear staining but that did show MS2B6 staining were rare, representing less

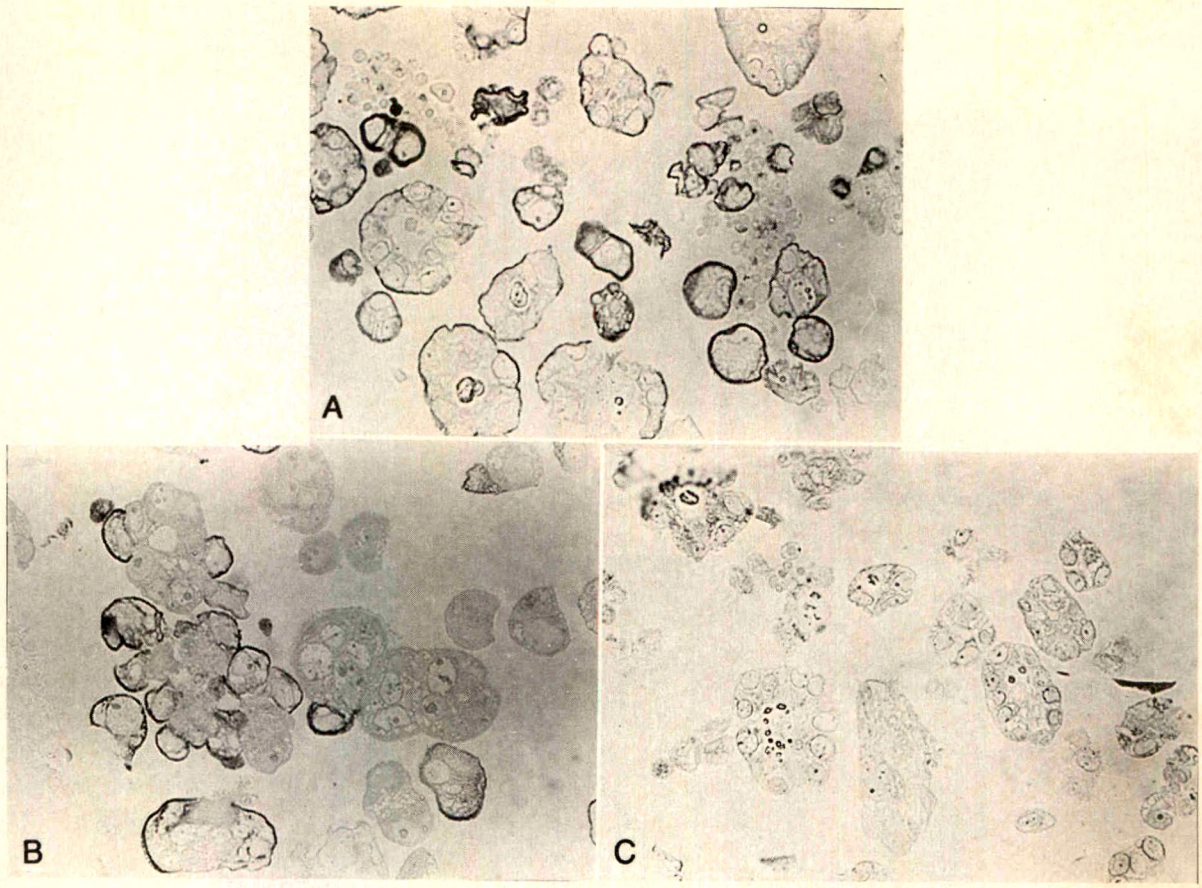


Fig. 5. Cellular localization of MS2B6 antigen. **A,** Methanol-acetone fixed ovarian cancer ascites cells (Bioton-labeled MS2B6 stain epoxy embedded; ×200); **B,** PLP-fixed ovarian cancer ascites cells (Biotin-labeled MS2B6 epoxy-embedded stain; ×200); **C,** Methanol-acetone fixed ovarian cancer ascites cells (Biotin-labeled negative control IgM epoxy-embedded stain; ×200).

than a few percent of the MS2B6 staining cells from any of the preparations studied. Although we cannot exclude the possibility that a rare viable cell does display the MS2B6 antigen on the cell surface, these results suggest that MS2B6 staining of fresh, unfixed cells is almost always restricted to damaged, nonviable cells and is not the result of surface staining of intact viable cells.

The immunoelectronmicroscopy experiments were performed to reveal the subcellular location of the bandlike staining deposits seen in the immunoperoxidase studies. Ascites tumor cells that had been methanol-acetone fixed and MS2B6 stained were chosen because in the light microscope nearly all of these cells demonstrated this ringlike deposit of DAB (Fig. 5, *A*). Fig. 7 shows electron micrographs of MS2B6-stained cells compared with control IgM-stained cells. Once again, a ringlike distribution of MS2B6 antigen is suggested. However, the layer of deposited DAB is clearly intracellular, beginning below the outer limits of the cell periphery and interdigitating with the peripheral cytoplasmic structures. Although the lipid bilayer structure of the plasma membrane is not consistently seen (presumably the result of the methanol-acetone fixation step), microvilli that serve as markers of the cell surface can be identified. The MS2B6 staining begins immediately beneath the level of these microvilli and extends into the peripheral cytoplasm in a bandlike layer.

CA 125 competition assay. MS2B6 was also tested for its ability to interfere with OC 125 in an enzyme-linked immunosorbent assay for CA 125. Although as little as 50 ng of unlabeled OC 125 inhibited the CA 125 enzyme-linked immunosorbent assay, MS2B6 in amounts up to 5 μg had no effect (data not shown). It is concluded that MS2B6 does not recognize the CA 125 epitope.

Comment

The human IgM MAb MS2B6 recognizes an antigen present in all ovarian carcinomas tested and in the majority of nonovarian adenocarcinomas. The antigen is present in trophoblastic tissue and in some fetal epi-

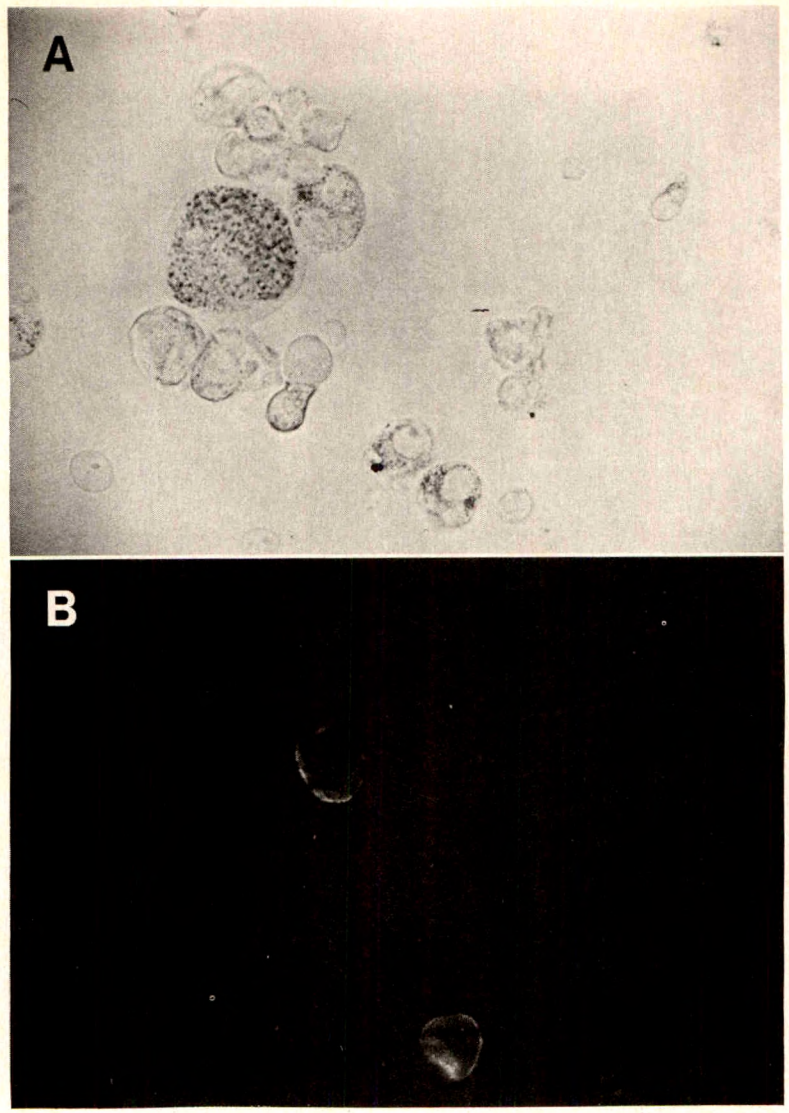


Fig. 6. MS2B6 antigen in unfixed ovarian cancer ascites tumor cells (Immunofluorescent stain). **A,** Light microscopy; **B,** Ultraviolet illumination (both same field, ×400).

thelia, but in the adult is restricted to normal, simple (nonsquamous) epithelia such as the fallopian tube, endometrium, endocervix, colon, bronchus, breast, sweat duct, and large renal ducts. The antigen was absent in normal peritoneum, stromal, or blood tissues, squamous epithelia, squamous cancers, sarcoma, melanoma, lymphoma, or malignant germ-cell tumors. The MS2B6 epitope was found to reside on polypeptides of approximately 38 and 44 kd, although some size heterogeneity was observed. A 60 kd band was observed but was not consistently present.

The MS2B6 MAb was originally detected by its ability to bind unfixed cryopreserved ovarian cancer ascites tumor cells with an immunofiltration technique.[13] This technique was selected to detect MAbs that recognized antigens on the tumor cell surface. However, the frozen-section immunoperoxidase results did not supply sufficient resolution to determine the cellular localization of MS2B6 antigen. The immunoperoxidase studies with ovarian cancer ascites tumor cells suggest that the MS2B6 antigen has a ringlike distribution near the cell periphery. However, many more cells were stained after permeabilization with methanol-acetone, suggesting that disruption of the cell membrane is required for staining by MS2B6. The initial immunofluorescence experiments also suggested a ring distribution of the MS2B6 antigen, but the propidium iodide counterstaining experiments clearly established that the MS2B6 staining pattern is rarely observed on intact viable cells, suggesting a cytoplasmic location for the MS2B6 antigen. The immunoelectronmicroscopic observations are consistent with those findings. The

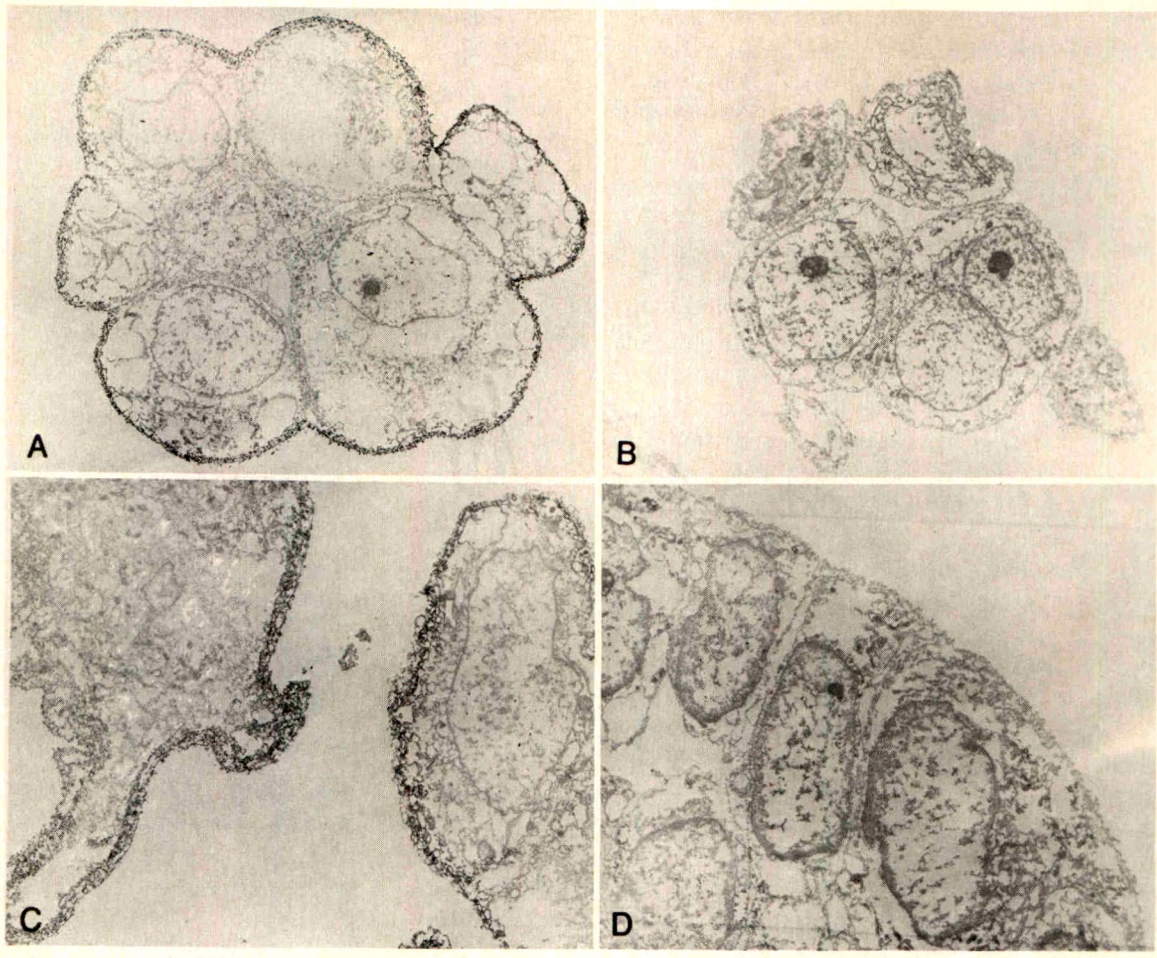


Fig. 7. Immunoelectronmicroscopy of MS2B6-stained ovarian cancer ascites cells. **A,** MS2B6-stained cells, ×1700; **B,** Control IgM-stained cells, ×1700; **C,** MS2B6-stained cells, ×2800; **D,** Control IgM stained cells, ×2800.

MS2B6 antigen is distributed in a bandlike layer of the peripheral cytoplasm in ascites tumor cells. Whether this distribution is the same for ovarian cancer tissues or normal epithelia that express the MS2B6 antigen is unknown at present.

The molecular size of the MS2B6 antigen, its tissue distribution, and its presence in the cytoplasm of ovarian cancer ascites tumor cells suggest that it could be a cytokeratin-type molecule. Human MAbs to cytokeratin-like antigens, described previously,[14, 24-26] are derived from lymphocytes of patients with adenocarcinomas of the breast, colon, and stomach. For example, human MAbs 16.88 and 28A32 were originally detected by surface staining of colon cancer cell cytospin preparations,[14] but subsequent immunofluorescence analysis with propidium iodide counterstaining suggested that the target antigens were actually cytoplasmic.[27] To our knowledge the bandlike distribution of the MS2B6 antigen in the peripheral cytoplasm of ovarian cancer ascites tumor cells has not been previously described for any of the cytokeratins, which typically display a filamentous structure throughout the entire cytoplasm of neoplastic cells.[28] The relationship between the MS2B6 antigen and the cytokeratin family is a subject of active investigation in our laboratories (Glasky MS, Yin A, Smith LH, et al., unpublished data).

The MS2B6 antigen does not appear to be identical to other cancer-associated antigens previously reported. A human IgM MAb to ovarian cancer that recognizes a predominantly nuclear antigen in ovarian cancer cells has recently been reported[29]; this pattern is quite distinct from the MS2B6 antigen distribution. A large number of ovarian cancer–associated antigens have been detected with mouse MAbs; many of these antigens possess some characteristics similar to the MS2B6 antigen, but distinct differences are recognized. For example, Miotti et al.[30] used mouse MAbs to detect ovarian cancer–associated antigens that have size characteristics similar to the MS2B6 antigen; however, unlike the MS2B6 antigen, they were not detectable after boiling in SDS. Mattes et al.[31-33] also used mouse MAbs to detect a series of ovarian cancer–associated antigens

that have size characteristics similar to the MS2B6 antigen but that are different in their behavior under reducing conditions. The competition-binding experiment presented here clearly demonstrates that MS2B6 failed to cross-react with the CA 125 epitope.

Our intention has been to use human hybridoma technology to study the human antibody response to gynecologic malignancy and to derive human MAbs of potential clinical utility. Human MAbs recognizing surface molecules on cancer cells are logical candidates for the development of imaging and therapeutic reagents and could be valuable alternatives to the more immunogenic mouse MAbs. The finding that the MS2B6 antigen is not a cell surface structure does not, however, completely eliminate the potential for clinical application of the human MAb MS2B6. In fact, monoclonal antibodies directed to intracellular antigens have been successfully used to image human tumor xenografts[34, 35] and have been shown to accumulate in damaged or necrotic tumor cells. The human MAbs 16.88 and 28A32 recognize intracellular antigens[27] and have been used successfully for localization of human colon cancer xenografts[36] and detection of metastatic disease in patients with colon cancer patients.[37] Uptake into tumor tissue of MAbs directed to intracellular antigens likely depends on the ubiquitous presence of some nonviable tumor cells. Normal healthy tissues that might express the same intracellular antigen avoid MAb uptake and thus potentially undesirable effects on imaging or therapy. We have used radioiodine-labeled MS2B6 given intraperitoneally for biodistribution studies in nude mice bearing human ovarian cancer cell line xenografts finding significant accumulation of labeled MS2B6 into intraperitoneal tumor deposits when compared with normal tissues.[38] These preliminary findings and the lack of significant toxicity or immune response to human MAbs administered to human subjects[37, 39-41] encourage further preclinical investigation of MS2B6 as an imaging or therapeutic agent.

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A randomized, placebo-controlled trial of betamethasone for the prevention of respiratory distress syndrome at 24 to 28 weeks' gestation

Thomas J. Garite, MD, Pamela J. Rumney, RNC, Gerald G. Briggs, BPharm, James A. Harding, MD, Michael P. Nageotte, MD, Craig V. Towers, MD, and Roger K. Freeman, MD

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Previous randomized controlled studies of corticosteroids for the reduction of respiratory distress syndrome have failed to demonstrate benefit in very early premature gestational age groups. A randomized, double-blind, placebo-controlled clinical trial of betamethasone given to mothers with intact membranes and threatened premature delivery between 24 and 28 weeks of pregnancy was conducted. Thirty-six patients were randomized to receive betamethasone, two doses of 12 mg, 24 hours apart, and 41 received placebo. No difference was found in the overall incidence of respiratory distress syndrome between the two groups (betamethasone vs placebo 0.55 vs 0.66) or in the incidence of respiratory distress syndrome in babies delivered between 1 and 7 days after the first dose of drug (betamethasone vs placebo 0.78 vs 0.88). Nor were there any differences observed in any measure of severity of respiratory distress syndrome between the groups. The neonatal death rates were also similar (betamethasone vs placebo 0.25 vs 0.24).The only difference seen was an unexpected reduction in the betamethasone group in the incidence of grades 3 and 4 intraventricular hemorrhage (betamethasone vs placebo 1/31 vs 9/36, $p = 0.01$). Therefore this study was unable to demonstrate any beneficial effect of corticosteroids in reducing respiratory distress syndrome at <28 weeks' gestation in spite of a sample size that had an 80% likelihood of detecting a 50% reduction in the incidence of respiratory distress syndrome with $p = 0.05$, which is the minimum reduction seen in virtually all randomized trials in other gestational age groups. (AM J OBSTET GYNECOL 1992;166:646-51.)

Key words: Corticosteroids, respiratory distress syndrome, prematurity

Liggins and Howie[1] first described the administration of maternally administered corticosteroids for the acceleration of fetal pulmonary maturity in 1972. Since that time numerous randomized controlled trials have confirmed the benefit of dexamethasone or betamethasone, which, when administered to patients with threatened premature delivery, substantially decreases the risk of surfactant-deficiency respiratory distress syndrome (RDS) in the preterm newborn. Nearly all trials have confirmed this benefit but certain subgroups of patients do not seem to benefit as much or apparently at all from this therapy. One such group is those who are delivered at <28 weeks of gestation. While there may be some theoretical reasons why corticosteroids might not benefit these very premature newborns, virtually all trials of such therapy have not included sufficient numbers of patients in this gestational age range to determine benefits. Therefore we undertook this randomized controlled trial of betamethasone in patients with threatened premature delivery (between 24 and 28 weeks' gestation).

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6/1/33043

Material and methods

All patients admitted to the perinatal service of Long Beach Memorial Women's Hospital with intact membranes between 24 weeks and 27 weeks 6 days of gestation who were in premature labor or otherwise judged likely to be delivered within the ensuing week, but not within 24 hours, were eligible for inclusion in the study. All patients were managed by the same group of physicians, which included perinatal attending physicians and fellows. The study was approved by the Human Subjects Review Committee, and all patients signed informed consent forms before randomization. Estimated gestational age was determined by a reliable date of last menstrual period with ultrasonographic

Table I. Demographic comparison

	Betamethasone (n = 33) (33 neonates)		*Placebo (n = 38) (40 neonates)*		*p Value*
Maternal age (yr)	26.3 ± 5.4		27.1 ± 6.0		NS
Gestational age at admission (wk)	25.5 ± 1.2		25.8 ± 1.3		NS
Cervical examination at admission					
0-2 cm	21	(64%)	23	(61%)	NS
3-4 cm	4	(12%)	11	(29%)	NS
>4 cm	1	(3%)	1	(3%)	NS
No cervical examination	7	(21%)	3	(8%)	NS
Parity					
0	14	(42%)	22	(58%)	NS
1	19	(58%)	16	(42%)	NS
Maternal transfer	25/33	(75%)	29/38	(76%)	NS
Contractions at admission					
Regular	20	(61%)	17	(45%)	NS
Irregular	8	(24%)	8	(21%)	NS
None	4	(12%)	13	(34%)	NS
Unknown	1	(3%)			
Admitting diagnosis*					
Premature labor	16	(48%)	22	(58%)	NS
Pregnancy-induced hypertension	1	(3%)	6	(16%)	NS
Vaginal bleeding	7	(21%)	7	(18%)	NS
IUGR, antepartum fetal distress	2	(6%)	2	(5%)	NS
Other	7	(21%)	1	(3%)	NS
Tocolytics (intravenous magnesium sulfate)	24/33	(73%)	34/38	(89%)	NS
Delivery interval (days)					
0-1	6	—	11	—	NS
2-7	13	—	17	—	NS
≥8	14	—	12	—	NS
Route of delivery					
Spontaneous vaginal	17	(52%)	18	(47%)	NS
Cesarean section	16	(48%)	20	(53%)	NS
Sex of live-born infants					
Male	18		14		NS
Female	15		26		
Birth weight (gm)	1242	± 678	1071	± 597	NS
Babies weighing 500-1000 gm	19/33	(58%)	22/40	(55%)	NS
Babies with low Apgar score (≤3 at 1 min, ≤6 at 5 min)	9/33	(27%)	16/40	(40%)	NS

*Some patients with more than one admitting diagnosis.

confirmation. When the estimated gestational age by sonogram differed by >14 days from the menstrual dates, the dates were revised to match the ultrasonography-estimated gestational age. Patients were randomized by members of the pharmacy section using a random number–generated, consecutive, sealed envelope system. Patients randomized to the study group received two doses of intramuscular Celestone (6 mg betamethasone acetate and 6 mg betamethasone phosphate) 24 hours apart. Patients randomized to the control (placebo) group received two doses of normal saline solution in the same quantity at 24-hour intervals. Both drug and placebo were prepared in the hospital pharmacy. Management was otherwise unaffected by inclusion in this study. Patients who received tocolytic therapy were given magnesium sulfate in 4 or 6 gm loading doses and maintained initially at 2 gm/hr adjusted to maintain contraction frequency at six or fewer per hour with serum levels not to exceed 7 mg/dl. Patients with ruptured membranes and clinical and/or laboratory evidence of infection, who had previously received any corticosteroids or had medical conditions contraindicating steroids, including diabetes, were excluded from this study.

Patients who had not been delivered 1 week after receiving the first dose, and who were judged still likely to be delivered in the next week, were given the same drug or placebo if the gestational age was still within the study inclusion range. Patients who had not been delivered after 28 weeks and were 1 week from their

last dose were allowed treatment with corticosteroids at the discretion of their attending physicians. Newborns were managed by attending neonatologists, and care was not altered by study participation. No neonate received surfactant therapy during the study period.

Outcome end points for the study included incidence and severity of RDS and neonatal survival. The diagnosis of RDS required clinical signs and tachypnea and retractions, supplemental oxygen therapy for at least 24 hours, and a chest x-ray film consistent with hyaline membrane disease. Secondary end points considered included maternal and neonatal infectious complications and other neonatal morbidity associated with prematurity, as well as length of neonatal hospitalization.

Statistical comparisons were made with t tests, χ^2 analysis, and Fisher's exact tests where appropriate.

Results

The study was conducted between December 1984 and May 1990. During this time 76 patients were successfully randomized, 37 to the betamethasone group and 39 to the placebo group. Of the 37 randomized to betamethasone, four were delivered elsewhere and were lost to follow-up; similarly, one patient was lost from the placebo group. Thus 33 received betamethasone and 38 placebo. All patients lost to follow-up had progressed beyond 29 weeks and would not have affected the analysis of outcome within 1 week of the last drug administered. Each group included three sets of twins. There were three stillbirths in the betamethasone group and one stillbirth in the placebo group. When twins were included and stillbirths were excluded, there were 33 neonates in the betamethasone group and 40 in the placebo group for the final neonatal analysis. Demographic and matching comparisons for the two groups are shown in Table I. As shown, there were no statistically significant differences between the two groups at the time of randomization in the interval to delivery, route of delivery, birth weight, or Apgar scores.

Of the 33 mothers in the betamethasone group, six were delivered <24 hours from the last dose of betamethasone, 13 at 2 to 7 days, and 24 after 7 days. Of the 40 randomized to placebo, eight were delivered in <24 hours, 17 between 2 and 7 days, and 12 after 1 week. Six of the betamethasone group and four of the placebo group received multiple courses of study drug or placebo (for betamethasone: two courses, four; three and four courses, one each; for placebo: two courses, one).

The incidence of maternal infections was not significantly different between the two groups. Chorioamnionitis occurred once in the betamethasone group and twice in the placebo group. Endometritis developed in 10 of 33 (30%) in the betamethasone group and five of 38 (13%) in the placebo group ($p = 0.07$). Correcting endometritis for route of delivery did not achieve statistical significance because this complication developed in seven of 16 cesarean sections in the betamethasone group and four of 20 in the placebo group ($p = 0.12$).

The incidence of RDS in the two groups is shown in Table II, including overall incidence, incidence after the exclusion of neonatal deaths within 1 hour of birth, and incidence divided by interval to delivery. As seen, in none of these analyses was a difference in the incidence of RDS observed between the two groups. In Table III the duration of intubation and the maximum inspiratory pressures, as indicators of severity of RDS, are compared between the two groups and analyzed, similarly to the incidence. Again no statistical differences in severity of RDS was noted between the betamethasone and placebo groups. There was no apparent difference between the two groups in the incidence of prolonged oxygen dependence (>30 days), either among all neonates (betamethasone vs placebo 9/33 vs 9/40) or among those with RDS (betamethasone vs placebo 9/20 vs 9/27).

Neonatal outcome is shown in Table IV, and as shown, no differences can be seen in the rates of stillbirths or neonatal deaths. Other major neonatal morbidities compared included the incidences of patent ductus arteriosus, intraventricular hemorrhage, and documented neonatal sepsis. There were no differences between the two groups in the incidence of patent ductus arteriosus, either in drug-treated or surgically treated groups or in the combined group (betamethasone vs placebo 23/33 vs 18/40) or in documented cases of neonatal-treated sepsis (betamethasone vs placebo 1 vs 3). There was, however, a substantial difference in the incidence of grades 3 and 4 intraventricular hemorrhage, with the betamethasone-treated babies having only one such case (3%) as compared with nine in the placebo group (25%), ($p = 0.012$). The incidence of grades 1 and 2 intraventricular hemorrhage was similar (betamethasone vs placebo 26% vs 25%). The difference in mean duration of neonatal hospital stay did not reach statistical significance (betamethasone vs placebo 52.8 vs 78.8 days, $t = 1.83$, $p = 0.072$).

The sample size achieved had an 80% chance of demonstrating a 50% reduction in the incidence of RDS with $p = 0.05$.

Comment

In spite of a frequent and consistent demonstration of benefit of maternally administered corticosteroids in reducing the incidence of RDS in other premature gestational age groups, the available randomized controlled trials have not shown benefit in patients treated between 24 and 28 weeks of pregnancy. There are two

Table II. Incidence of RDS

	Betamethasone		*Placebo*		
	No.	*%*	*No.*	*%*	*p Value*
All patients	21/33	61	28/40	68	NS
Excluding immediate neonatal death	18/30	60	24/36	67	NS
Interval to delivery (days)					
0-1	6/6		8/8		NS
2-7	10/13		15/17		NS
>7	5/14		5/12		NS

Table III. Severity of RDS

	Intubation (days)		*Peak inspiratory pressure (mm Hg)*	
	Betamethasone	*Placebo*	*Pressure betamethasone*	*Placebo*
All living neonates	36.4 + 32 (n = 14)	32.6 + 40 (n = 19)	21.3 + 9 (n = 19)	28.5 + 11 (n = 24)
Interval to delivery 2-7 days	40.5 + 39 (n = 9)	42.3 + 48 (n = 10)	17.5 + 4 (n = 10)	25.3 + 10 (n = 10)
Minus 1 outlier per group	27.8 + 16 (n = 8)	27.1 + 16 (n = 9)		

All comparisons between bethamethasone and placebo showed no statistically significant differences.

possible reasons for this apparent lack of benefit. One is that this group is physiologically different, and thus the therapeutic response differs, compared with that of older gestational age groups. There are indeed reasons to expect that this may be the case. Corticosteroids are thought to promote fetal lung maturity through a number of mechanisms. The primary one is thought to be increased production and release of lung surfactant.[2, 3] Although steroid receptors are present in the type II alveolar cells, which produce surfactant as early as 12 weeks, there may be a quantitative difference in receptors as pregnancy progresses.[4] The type of surfactant produced also seems to be critical. The more mature fetus produces a disaturated phosphatidylcholine through the choline incorporation pathway. This pathway, which includes a substantial number of different enzymes thought to be affected by corticosteroids, may not be available or quantitatively as responsive in these very early gestations where other pathways for the production of surfactant may be dominant and may not be as responsive to corticosteroids.[5] Some studies suggest that steroids may work through structural changes in alveolar architecture, producing alterations in lung collagen and elastin.[6, 7] Since the early gestation lung may be more tubular and less saclike or alveolar, the lung tissue may not be as responsive to the effects of corticosteroids.

The other possible explanation is that betamethasone does lower the incidence of RDS, albeit at possibly less effective rates, but that the numbers of subjects in published study and control groups have been insufficient to demonstrate a difference. Analysis of the literature on this question is difficult, because several studies combine gestational ages or birth weights into larger groups, such as <31 weeks.[8-10] Indeed, in the review by Crowley et al.,[11] which included a meta-analysis of 12 controlled trials of corticosteroids including >3000 participants, the authors concluded that seven trials evaluating the effects of gestational age showed "an unambiguous and important reduction in the risk of respiratory morbidity among babies born at less than 31 weeks gestation." If one limits review to those specifying <29 weeks and/or ≤1000 gm or those in which data can be extracted from tables within the article, the actual number of patients in all previous individual randomized controlled trials at <29 weeks is actually quite small, the largest being that of Doran et al.[12] with 27 total patients. Even combining available randomized controlled trials gives a total of only 109 patients, all of whom showed no benefits with corticosteroid therapy.[1, 12-15] There have been several nonrandomized studies in this very premature gestational age group, where the comparison was made with control subjects who did not receive steroids for various reasons. In these studies there did appear to be less RDS in the steroid-treated patients.[16-18] Caution must prevail in interpreting these studies because the control subjects are usually not comparable. Control subjects usually re-

Table IV. Neonatal outcome

	Betamethasone		*Placebo*		
	No.	*%*	*No.*	*%*	*p Value*
All patients					
Stillbirth	3	9	1	2	NS
Neonatal death	9	25	11	24	NS
Alive at discharge	24	66	29	68	NS
Patients delivered at >2 and <7 days					
Stillbirth	3	19	0	0	NS
Neonatal death	6	38	4	24	NS
Alive at discharge	7	44	13	76	NS

ceived β-sympathomimetic tocolytics less often than did steroid-treated patients, which may accelerate lung maturity and often included more babies with fetal distress necessitating earlier delivery, which may predispose to more cases of RDS and other nonequivalent factors.

The current study was unable to demonstrate any beneficial effect of betamethasone in reducing the incidence or severity of neonatal respiratory distress, including other associated complications such as bronchopulmonary dysplasia. Nor were there any significant trends suggesting that a larger sample size would likely have demonstrated such a benefit. While certainly not large, the total of 76 patients randomized in this study is by far the largest sample size in any single study. In fact, the sample size achieved in this study gave an 80% chance of demonstrating a 50% reduction in the incidence of RDS, which is the reduction rate in virtually all other studies in more advanced gestational ages.

Because of the strict inclusion criteria and limited gestational ages included, this study was conducted over a relatively long time period. Consequently the results of this study may be somewhat less clinically relevant than those at the time the study was originally designed. More recent studies of the prevention of RDS often include neonatal surfactant therapy. It appears preliminarily that corticosteroids may act synergistically with surfactant therapy to achieve benefit, even in the extremely premature babies included in this study.[19, 20]

A somewhat surprising reduction in the incidence of more severe grades of intraventricular hemorrhage was seen in the betamethasone-treated neonates. This would not have been a surprise if there were other differences between the two groups in the rates of RDS, severity of RDS, or an unexpected difference in Apgar scores. Such morbidities are known to increase the risk of intraventricular hemorrhage, but, other than the betamethasone itself, there are no other differences in this study between the treated and control groups that might explain the observed difference in the rate of severe intraventricular hemorrhage. However, it should be acknowledged that the only comparison between the two groups for the possible occurrence of perinatal asphyxia is by Apgar scores and other associated neonatal morbidity. Umbilical cord blood gas levels were not routinely measured at the time this study was begun; thus valid comparisons cannot be provided. Betamethasone itself may have some stabilizing effects on the fragile germinal matrix capillaries or other unknown protective mechanisms and this observed difference may be real. Previous randomized trials of corticosteroids have not shown a reduction over controls in rates of intraventricular hemorrhage. One study evaluating obstetric factors that affect rates of intraventricular hemorrhage observed a reduction in corticosteroid-treated mothers.[21] In a study evaluating the effects of antenatal vitamin K therapy on intraventricular hemorrhage, an independent beneficial effect of maternal corticosteroids was also seen.[22] In the review and meta-analysis by Crowley et al.,[11] a moderate reduction in the incidence of intraventricular hemorrhage can be seen by combining available controlled studies. Again, is this from less RDS with a secondary reduction in intraventricular hemorrhage or from some direct effect on the fetal germinal matrix? No definitive answer can be given. From the current study we are reluctant to draw any real conclusions regarding betamethasone and intraventricular hemorrhage for two reasons. First, the effect on intraventricular hemorrhage was not one of the original hypotheses, because such a beneficial effect on intraventricular hemorrhage had not been suggested when this study was started in 1984; thus the difference may be a type I error. Furthermore, the incidence of grades 3 and 4 intraventricular hemorrhage in the institution where this study was done, during this time period and at these gestational ages, ranged from 8% to 10%. Thus the 25%

incidence seen in the placebo group appears to be unusually high, possibly explained by chance alone.

The trend toward more instances of postpartum endometritis in patients receiving betamethasone is certainly a concerning one and difficult to explain. This complication has not been described in other studies of corticosteroids in patients with intact membranes. However, occult intraamniotic bacterial infection in patients in premature labor has been shown to be more common in earlier premature gestational ages than later ones.[23]

Thus a double-blind, randomized, placebo-controlled trial was conducted in patients with threatened premature delivery (before 28 weeks of gestation). In this study we were unable to demonstrate evidence of benefit in reducing the incidence or severity of RDS in spite of a sample size likely to demonstrate the magnitude of benefit seen in older gestational age groups. This study did not evaluate the utility of corticosteroids in the presence of neonatal surfactant therapy and therefore is not relevant to this more contemporary practice. The reduction of intraventricular hemorrhage seen in this study is provocative and has been suggested by a few previous investigators; thus further studies looking at this therapy for the expressed purpose of reducing intraventricular hemorrhage in these very premature babies may be warranted.

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Gonadotropin and ovarian hormone dynamics in luteal phase defects

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Blood samples were obtained during early follicular, periovulatory, and luteal phases in four women with out-of-phase endometrial biopsy specimens and four normal controls. In the study cycle, follicular development was evaluated and a late luteal phase endometrial biopsy was performed in each subject. Area under the luteal phase progesterone curve positively correlated with degree of maturity of the endometrial biopsy. Peak serum estradiol, maximum follicular diameter, and both immunoactivity and bioactivity of the preovulatory luteinizing hormone and follicle-stimulating hormone surges were similar in the luteal phase defect cycles as compared with normal cycles. Likewise luteinizing hormone bioactivity in the luteal phase of the luteal phase defect cycles was similar to that of normals. These data show that the immunoactivity and bioactivity of periovulatory and luteal phase gonadotropins may be normal in luteal phase defect cycles. (Am J Obstet Gynecol 1992;166:652-7.)

Key words: Luteal phase defect, gonadotropin, progesterone, ovulation.

Although luteal phase defect was first described 40 years ago,[1] the precise pathophysiologic characteristics of the defect remain unclear. Luteal phase defect has been suggested to be a result of many mechanisms, such as hypothalamic-pituitary dysfunction manifested by insufficient follicle-stimulating hormone (FSH) stimulation of the developing follicle,[2-5] an inadequate luteinizing hormone (LH) or FSH surge,[6-8] an attenuation of LH immunoactivity and bioactivity during the midcycle surge,[6] or a lack of proper luteal phase LH support of the corpus luteum progesterone synthesis.[9] Because the preovulatory FSH surge may play an important role in induction of LH receptors on granulosa cells in preparation for luteal phase progesterone production,[8] inadequate bioactivity of the FSH molecule during the early follicular phase or the preovulatory surge could result in poor corpus luteum response to luteal phase LH secretion. In the present study we sought to more clearly characterize the dynamics of pituitary FSH and LH secretion during the periovulatory period in patients with luteal phase defects.

Material and methods

Eight normoprolactinemic women with infertility who had a >2-day lag in a late luteal phase endometrial biopsy were included in the study. Monitoring of hypothalamic-pituitary-ovarian dynamics was started in a following menstrual cycle when a confirmatory biopsy was planned. Venous blood samples were collected daily in the early follicular phase, twice daily in the preovulatory period, and every other day during the luteal phase. Growth of the dominant follicle was monitored with a 5 MHz convex-array vaginal ultrasonographic transducer (Corometrics Medical Systems, Inc., Wallingford, Conn.) twice daily in the periovulatory period until verification of follicular rupture. This was evidenced by disappearance or flattening of the follicle with a decrease in the mean diameter. Two to five days after the LH surge, a culdocentesis was performed to confirm ovulation by determination of peritoneal fluid progesterone concentrations. Endometrial biopsy was performed 10 to 12 days after ovulation. Samples were obtained from the anterior uterine fundus with a Novak curette.

Five controls were selected from a group of women with either proven fertility or male factor infertility, normal menstrual histories, and no previous endometrial biopsies. Monitoring of the study cycle was carried out exactly as in the luteal phase defect group. None of the subjects took hormone supplements or antiinflammatory agents during the study.

Serum and peritoneal fluids were stored at −20° C until assayed. Estradiol concentration was assayed by a commercially available radioimmunoassay kit (Direct ^{125}I Estradiol, Pantex, Santa Monica, Calif.) as was serum progesterone (Coat-a-Count Progesterone, Diagnostic Products, Los Angeles). Immunoactive LH and FSH were determined by a double-antibody radioimmunoassay with LER-907 as the reference stan-

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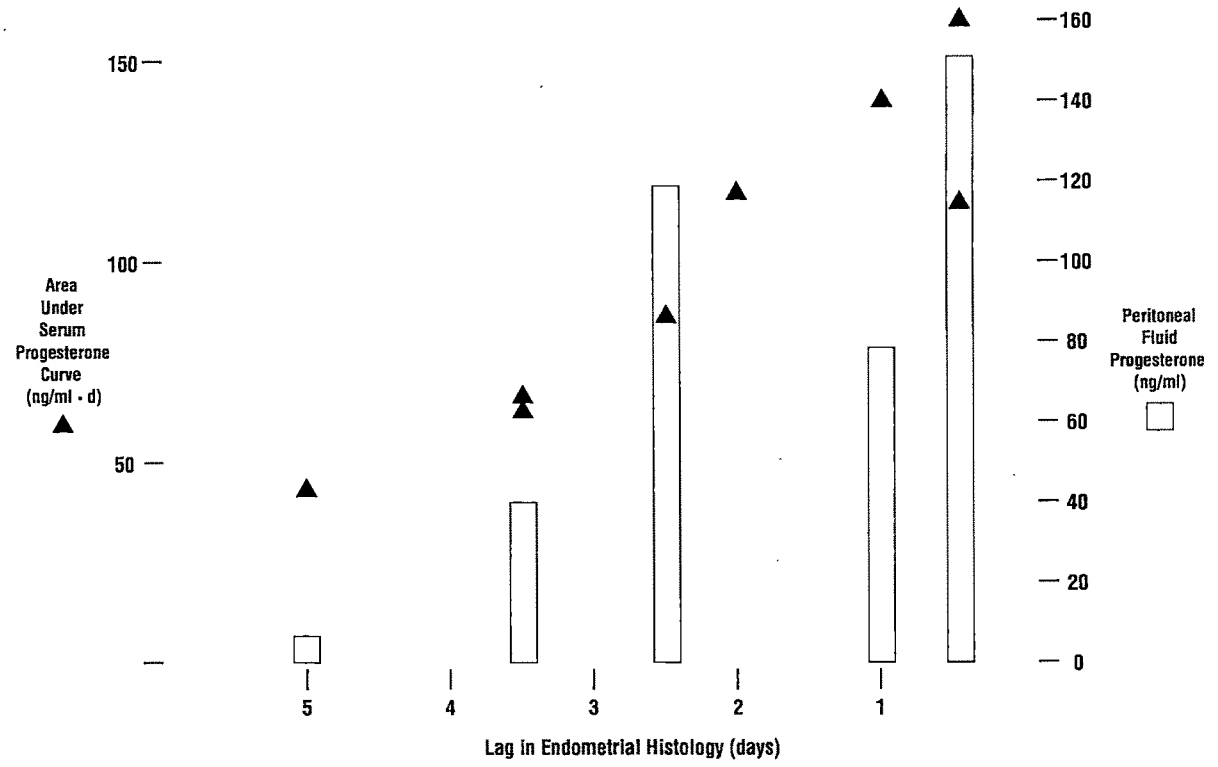


Fig. 1. Relationship of endometrial histology to peritoneal fluid progesterone concentration and area under the luteal phase progesterone curve.

dard.[10, 11] Intraassay and interassay coefficients of variation for the immunoactive LH assay were 5.3% and 11.6% and those for the immunoactive FSH assay were 5.3% and 8.8%, respectively. The sensitivity for the immunoactive LH assay was 2 ng/tube and for the immunoactive FSH assay 12 ng/tube.

Bioactive LH was determined by the mouse interstitial cell in vitro bioassay[12, 13] with LER-907 as the reference standard. The coefficients of variation were 9% and 20% within and between assay, respectively. Parallelism and potency estimates were calculated with W.E. Bridson's computer program. Bioactive FSH was measured by the rat granulosa cell aromatase in vitro bioassay with LER-907 as the reference standard.[14] This assay measures FSH-induced production of estradiol from dispersed granulosa cells obtained from diethylstilbesterol-primed rats. Each serum specimen was pretreated with polyethylene glycol to remove inhibitory substances, and duplicates were assayed in three different concentrations. The intraassay and interassay coefficients of variation were 12.5% and 14.8%, respectively. Parallelism and potency estimates were calculated with W.E. Bridson's computer program. Each endometrial biopsy was interpreted by two reproductive endocrinologists according to the criteria of Noyes et al.[15] in a blinded manner before performance of the luteal phase progesterone assays.

Areas under curves were calculated by Simpson's rule. Statistical significance was calculated by the Student t test.

Results

Repeat endometrial biopsies in five of the eight subjects in the study group were histologically in phase; therefore those subjects were excluded from further analysis. Four of the five controls had an in-phase biopsy; however, one was out of phase. Since her complete cycle was evaluated, which culminated in an out-of-phase biopsy, she was included in the luteal phase defect group. This resulted in four subjects with luteal phase defects defined as an endometrial biopsy that was >2 days out of phase, compared with a group of four controls with normal biopsies. One of the four luteal phase defect cycles had a luteinized unruptured follicle, as determined by early postovulatory peritoneal fluid progesterone concentration, which was equal to the level in the serum (Table I).

The mean area under the luteal phase progesterone curve was significantly less in the luteal phase defect group (67 ± 9 ng/ml · d) versus the normal group (132 ± 11 ng/ml · d) $p < 0.005$. This also correlated positively with peritoneal fluid progesterone levels ($p < 0.05$) and the degree of maturity of the endometrial biopsy ($p < 0.001$) (Fig. 1).

There was a trend toward lower immunoactive FSH levels on cycle days 1 to 3 in the luteal phase defect

Table I. Comparison of individual areas under luteal phase progesterone curves, peritoneal fluid progesterone, and ultrasonographic findings

Subject	*Area under luteal phase progesterone curve [(ng/ml) · d]*	*Peritoneal fluid progesterone (ng/ml)*	*Ovulation by ultrasonography*
Luteal-phase defect			
1	44	7.2*	+
2	85	118	+
3	70	40	+
4	68	40	+
Normal endometrial biopsy			
5	114	—†	+
6	139	79	+
7	158	151	+
8	116	—†	+

*Equal to serum progesterone concentration.
†Unable to obtain fluid.

Table II. Peak gonadotropin and estradiol values and follicular diameters in luteal phase defect and control subjects during the preovulatory gonadotropin surge and luteal phase length (mean ± SEM)

	Control (n = 4)	*Luteal phase defect (n = 4)*
Peak immunoactive LH (ng/ml)	319 ± 172	243 ± 74
Peak bioactive LH (ng/ml)	2446 ± 1035	2348 ± 157
Peak immunoactive FSH (ng/ml)	788 ± 102	776 ± 63
Peak bioactive FSH (ng/ml)	814 ± 112	766 ± 63
Serum progesterone at LH surge (ng/ml)	0.61 ± 0.13	0.78 ± 0.13
Peak serum estradiol (pg/ml)	324 ± 42	273 ± 37
Maximum follicular diameter (mm)	21 ± 2.8	17 ± 0.3
Luteal phase length (days)	14 ± 0	11.3 ± 1.5

group than in the normal subjects, but this did not reach statistical significance (307 ± 12 ng/ml vs 472 ± 140 ng/ml). Early follicular phase bioactive FSH levels were not assayed. Immunoactive LH levels were similar during the same time period (64 ± 0.5 ng/ml vs 50 ± 7.6 ng/ml). Comparison of gonadotropin levels during the preovulatory surge in the two groups showed no difference in peak immunoactive LH, bioactive LH, immunoactive FSH, or bioactive FSH (Table II). At the onset of the surge, progesterone levels were similar in the two groups, as were peak estradiol levels and maximum follicular diameter (Table II). Likewise, there were no differences in areas under the immunoactive or bioactive LH or immunoactive or bioactive FSH curves between the two groups during the surge (Fig. 2). Although the luteal phase of the subject with luteinized unruptured follicle had a 9-day luteal phase, whereas the other subjects with luteal phase defect had 12-day luteal phases, midcycle hormonal parameters were similar (Table III).

Although the subjects with normal luteal phase endometrial biopsies had similar luteal phase immunoactive and bioactive LH levels, there was marked individual variation noted among three of the subjects with luteal phase defect, resulting in no statistically significant differences between those three subjects and a group of three controls (Fig. 3).

Comment

Soules et al.[6] have shown luteal phase defects to be associated with abnormally low immunoactive LH and bioactive LH levels at the time of the midcycle surge and low bioactive LH levels in the luteal phase. This suggests that luteal phase defect is a consequence of poor gonadotropin stimulation of a corpus luteum that may otherwise have normal steroidogenic potential. Because granulosa cells develop the capacity to synthesize and secrete progesterone at the time of the LH surge but do not secrete it in response to luteal phase LH pulses,[16] a faulty LH surge could lead to deficient progesterone production by luteinized granulosa cells. Additionally, low luteal phase bioactive LH levels would be expected to result in low progesterone production by the LH-responsive luteinized theca cells.

Alternatively, others have shown that this situation may not be present in some women with luteal phase defect who develop luteal phase inadequacy in spite of normal midcycle[17] and luteal phase[5] gonadotropin secretion. This may be due to poor response at the level of the corpus luteum or inability of the endometrium

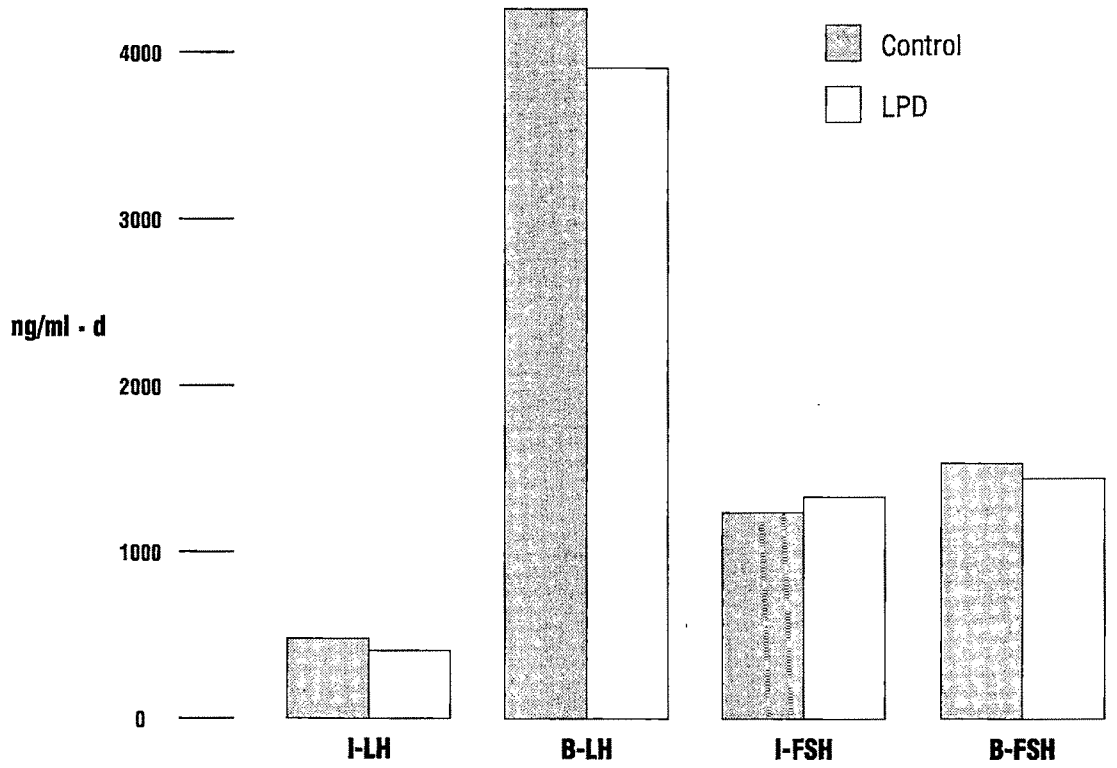


Fig. 2. Areas under midcycle gonadotropin surge curves. *LPD,* Luteal phase defect; *I-LH,* immunoactive LH; *B-LH,* bioactive LH; *I-FSH,* immunoactive FSH; *B-FSH,* bioactive FSH.

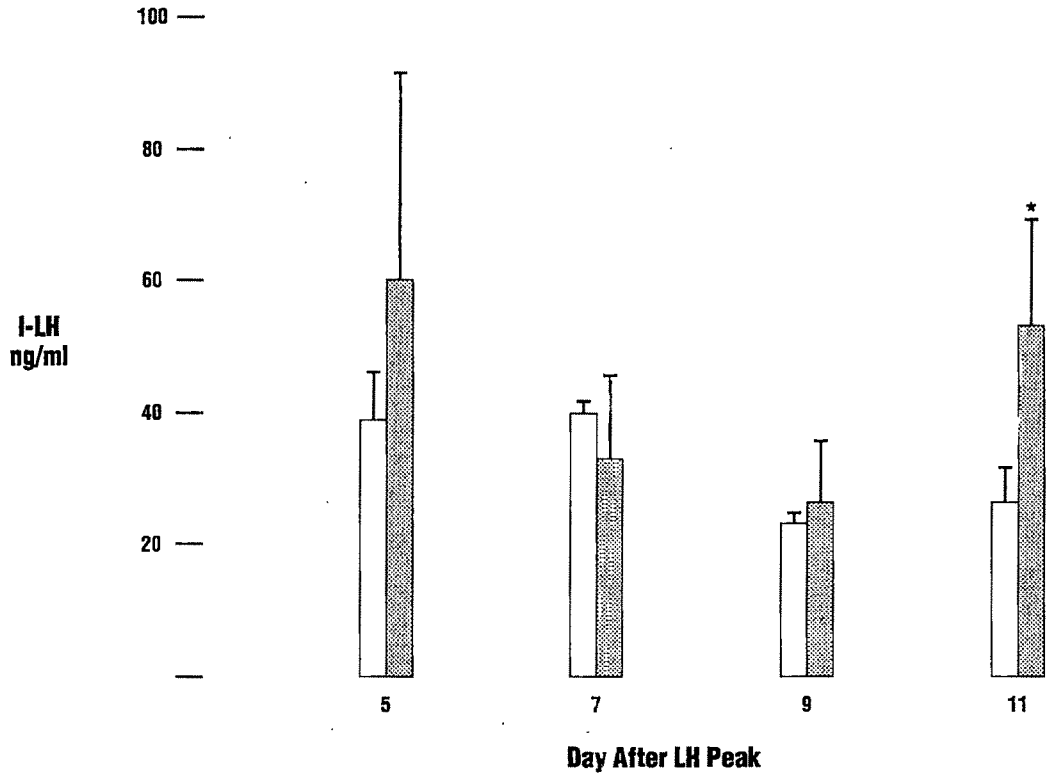
Table III. Midcycle hormone levels in subjects with luteal phase defect (mean ± SEM)

	Luteinized unruptured follicles	*Ovulatory cycles*		
Peak immunoactive LH (ng/ml)	196	156	159	462
Peak bioactive LH (ng/ml)	2074	2422	2135	2760
Peak immunoactive FSH (ng/ml)	790	790	610	913
Peak estradiol (pg/ml)	300	314	188	314

to respond to normal levels of progesterone because of a receptor defect. In spite of the small number of subjects in our study, by performing a thorough evaluation of midcycle and luteal-phase gonadotropin immunoreactivity and bioactivity by increasing the frequency of sampling, we were able to demonstrate gonadotropin values, in subjects with luteal phase defects, that did not deviate significantly from normal subjects. In fact, our data suggest heterogeneity in the etiology of luteal phase defect, suggesting that its origin may not necessarily be found at the level of the hypothalamic-pituitary axis in each case. This heterogeneity may account for the discrepancy between our findings and those of Soules et al.[6]

The finding of a correlation between luteal phase progesterone levels and endometrial secretory maturation demonstrated that the endometrium was capable of responding to progesterone in a quantitative fashion in the subjects of this study. This implies normal progesterone receptor function and suggests that the mechanism responsible for luteal phase defects in these subjects was not located at the level of the endometrium. The defect would seem to be localized to the ovary after the midfollicular phase, more specifically to the corpus luteum as women with luteal phase defects had normal periovulatory and luteal phase bioactive and immunoactive LH levels when sampled twice daily, although more frequent sampling would be helpful to confirm these findings because of the pulsatile nature of LH release throughout the menstrual cycle. The presence of a luteinized, unruptured follicle in one luteal phase defect cycle demonstrates that the two conditions can coexist but fails to demonstrate a causative relationship.

There was a trend toward lower early follicular phase immunoactive FSH values in the subjects with luteal



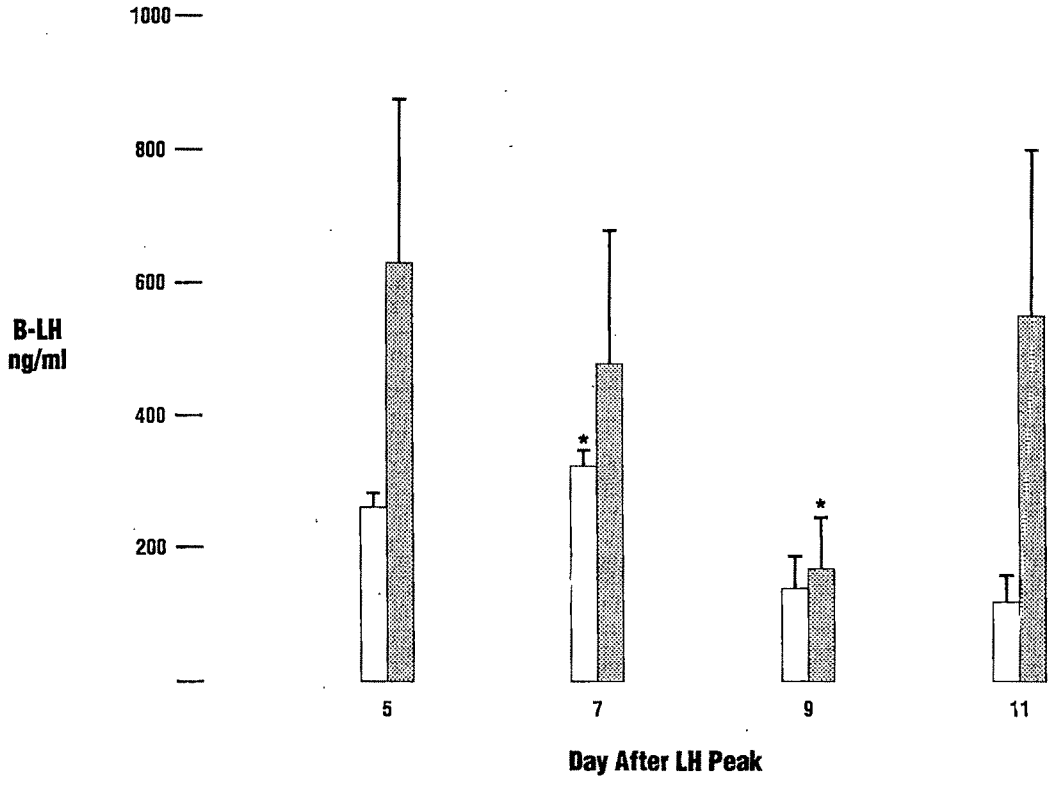


Fig. 3. Luteal phase LH values (mean ± SEM).

phase defect, but this did not reach statistical significance. Evaluation of a larger group of subjects with shorter sampling intervals would be more likely to identify any real difference. However, because of its relatively long half-life, the variation in serum immunoactive FSH concentration is less marked than that of immunoactive LH.[18] Follicular development, as measured by mean maximum follicular development and peak estradiol levels, was similar in the two groups.

The midcycle FSH surge may augment luteal phase corpus luteum responsiveness to LH by inducing its synthesis receptors on granulosa cells. Because luteinized granulosa cells synthesize and secrete progesterone during the first 10 days of the luteal phase,[16] luteal phase inadequacy in the face of normal luteal phase LH levels could be due to a poor midcycle FSH surge.[8] Our data revealed no difference in peak bioactive or immunoactive FSH levels between the normal and luteal phase defect groups, however. Likewise there was no difference in the area under the bioactive or immunoactive FSH curves between the two groups.

As previously observed and reported,[6, 8, 9] luteal-phase progesterone levels, calculated as the area under the curve, were lower in the luteal phase defect subjects in our study. These subjects also demonstrated lower progesterone concentrations in the postovulatory peritoneal fluid, suggesting that suboptimal ovarian progesterone secretion may be manifested as early as the periovulatory period. To our knowledge this is the first report of low peritoneal fluid progesterone concentrations in luteal phase defect cycles.

These data together with that from other studies[2-9, 19, 20] show that luteal phase defect may result from several conditions, including dysfunctional gonadotropin secretion, poor ovarian response to normal gonadotropin secretion, or inadequate endometrial secretory maturation in spite of normal ovarian progesterone secretion. An attempt to attribute the cause of all cases of luteal phase defect to only one of these mechanisms may be erroneous. Further investigation into early follicular phase and midcycle gonadotropin secretion and peritoneal fluid progesterone accumulation in normal and luteal phase defect cycles may enhance our understanding of this controversial condition.

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Serial thoracic versus abdominal circumference ratios for the prediction of pulmonary hypoplasia in premature rupture of the membranes remote from term

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OBJECTIVES: The purpose of this study was to evaluate the fetal thoracic versus abdominal circumference ratio in the prediction of pulmonary hypoplasia after preterm premature rupture of the membranes.

STUDY DESIGN: A standard curve of thoracic versus abdominal circumference ratio versus gestational age was derived from 120 uncomplicated pregnancies. Thoracic versus abdominal circumference ratio and amniotic fluid estimations were obtained serially in 16 women with premature rupture of the membranes at <26 weeks' gestation.

RESULTS: An abnormal thoracic versus abdominal circumference ratio predicted lethal pulmonary hypoplasia in six infants. A progressive decline in thoracic versus abdominal circumference ratio was identified in all of these patients. Six of eight women with persistent oligohydramnios had abnormal thoracic versus abdominal circumference ratios and lethal pulmonary hypoplasia. Two surviving infants had restriction deformities and pulmonary complications.

CONCLUSIONS: In the setting of premature rupture of the fetal membranes at <26 weeks, serial thoracic versus abdominal circumference ratio measurement is a reliable predictor of pulmonary hypoplasia. Persistent oligohydramnios is ominous because of the risk of restriction deformities and pulmonary complications in the survivors. (Am J Obstet Gynecol 1992;166:658-63.)

Key words: Pulmonary hypoplasia, prenatal diagnosis, premature rupture of fetal membranes

Pregnancy complicated by premature rupture of the fetal membranes before 26 weeks' gestation carries a significant risk of fetal and neonatal morbidity and mortality. Taylor and Garite[1] reported on the outcome of 53 cases of premature rupture of fetal membranes before fetal viability after expectant management. Beydouin and Yasin[2] identified a perinatal survival rate of 22% in infants of 70 women with premature rupture of fetal membranes before 28 weeks' gestation, 29.4% of 17 surviving infants were physically and developmentally normal at 6 months of age. Moretti and Sibai[3] demonstrated a 68% perinatal mortality, when premature rupture of fetal membranes occurred before 26 weeks' gestation, and an 87% mortality, when premature rupture of fetal membranes occurred before 23 weeks' gestation. In that study, 33% of the surviving infants had developmental abnormalities.

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Retrospective analysis of 50 patients treated at the University of Ottawa with premature rupture of fetal membranes that occurred between 16 and 26 weeks indicated that prolonged maternal hospitalization was common, the mean stay was 29.2 days. Chorioamnionitis developed in 42% of patients. Thirty-six percent of live-born infants died of pulmonary complications; five of these (15.2%) died of pulmonary hypoplasia. Only 16 of 55 infants, including 5 sets of twins (29.1%) survived, three of whom demonstrated serious neurologic handicaps at 1 year of age as a result of intraventricular hemorrhage.

Pulmonary hypoplasia is a major contributor to neonatal mortality in infants of women experiencing premature rupture of fetal membranes before 26 weeks' gestation. Many of these women pursue expectant management. Several clinical tests have been suggested to predict pulmonary hypoplasia, however, findings have not been consistent.

The purpose of this study was to create a standard curve for the thoracic circumference versus abdominal circumference ratio in a middle-class Caucasian population, to determine the value of serial thoracic versus abdominal circumference ratio measurements in the prediction of pulmonary hypoplasia and neonatal death in infants whose mothers experience premature

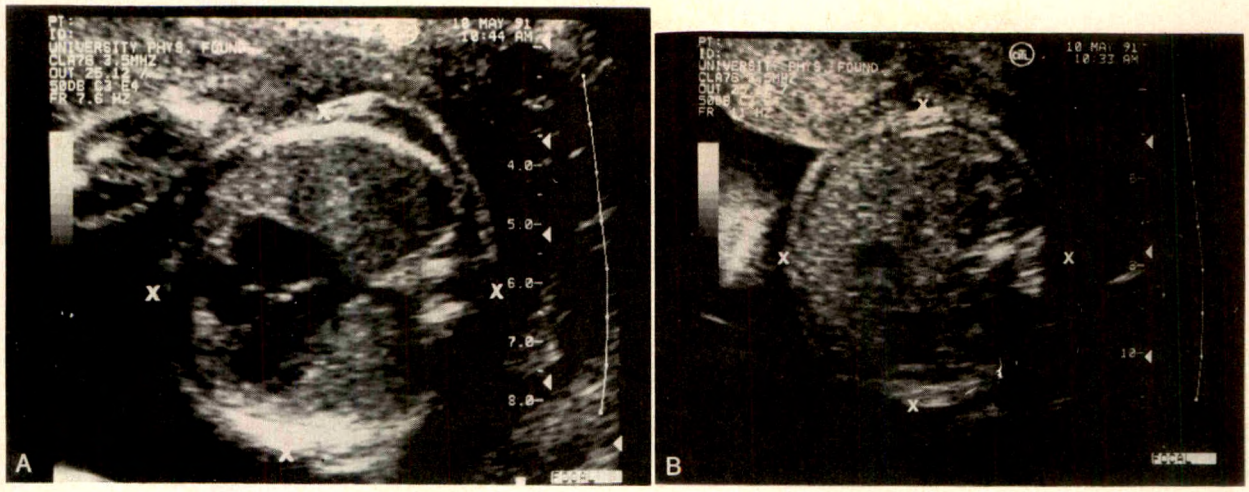


Fig. 1. A, Cross-sectional sonogram of fetal chest at level of four-chamber view of heart. **B,** Cross-sectional sonogram of fetal abdomen at level of bifurcation of umbilical vein.

rupture of fetal membranes before 26 weeks' gestation, and to determine the value of qualitative amniotic fluid volume assessment in the prediction of perinatal outcome in this population.

Material and methods

One hundred twenty women with secure dates based on menstrual history and/or early ultrasonography and appropriate growth for gestational age underwent ultrasonographic measurements of the fetal thoracic and abdominal circumferences. Measurements of the thoracic circumference were performed in the transverse plane of the fetus at the level of the four-chamber view of the heart (Fig. 1, *A*). The fetal abdominal circumference was measured in the transverse plane at the level of the insertion of the umbilical vein (Fig. 1, *B*). The thoracic versus abdominal circumference ratio was obtained for each fetus, and a scatter plot of thoracic versus abdominal circumference ratio versus gestational age was created. Simple linear regression was performed with the method of least squares. Confidence intervals that were based on ±2 SDs were applied between 14 and 40 weeks' gestation (Figs. 2, 3).

Between October 1, 1984 and December 31, 1987, 17 patients with premature rupture of fetal membranes that occurred before 26 weeks' gestation were referred to the high-risk unit at the Ottawa Civic Hospital for evaluation and management. Patients who had confirmed membrane rupture were initially evaluated 1 week after hospitalization to treat premature rupture of fetal membranes. Ultrasonographic assessment was performed to rule out associated fetal anomalies (particularly urogenital anomalies that might lead to persistent oligohydramnios and pulmonary hypoplasia). Patients were restricted to bed rest at home from the time of diagnosis to that of fetal viability and were then admitted to the hospital from 26 weeks' gestation until delivery. Fetal surveillance consisted of daily fetal movement charting, biweekly nonstress testing, and biweekly biophysical profile scoring performed weekly from 26 weeks' gestation.

Serial ultrasonographic measurements that included obtaining the thoracic versus abdominal circumference ratios were performed at 1- to 2-week intervals and were correlated to fetal outcome and autopsy data. Subjective assessment of amniotic fluid volume was performed at each scan. Amniotic fluid volume was considered normal if there was one pocket that measured at least 3 cm in the vertical axis. Neonatal outcome was reviewed with respect to perinatal pulmonary morbidity and mortality. Pulmonary hypoplasia was defined as a lung weight/body weight ratio of 0.012 or less.[4]

Results

Sixteen of 17 women studied suffered membrane rupture immediately before hospitalization. One woman who was first assessed at 23 weeks' gestation (thoracic versus abdominal circumference ratio 0.71) had prolonged membrane rupture before admission. All patients had had ultrasonography within 2 weeks before delivery.

After counseling, 16 of 17 patients elected to continue their pregnancies. Thirteen infants were delivered after 26 weeks' gestation, 8 of these 13 (62%) survived. None of the three infants who were delivered before 26 weeks survived. The mean time from premature rupture of fetal membranes to delivery was 61.8 days, the range was 31 to 152 days. The final thoracic versus abdominal circumference ratio for each infant before delivery was compared to the standard curve for our population (Fig. 2). The six infants with pulmonary hypoplasia predicted by an abnormally low tho-

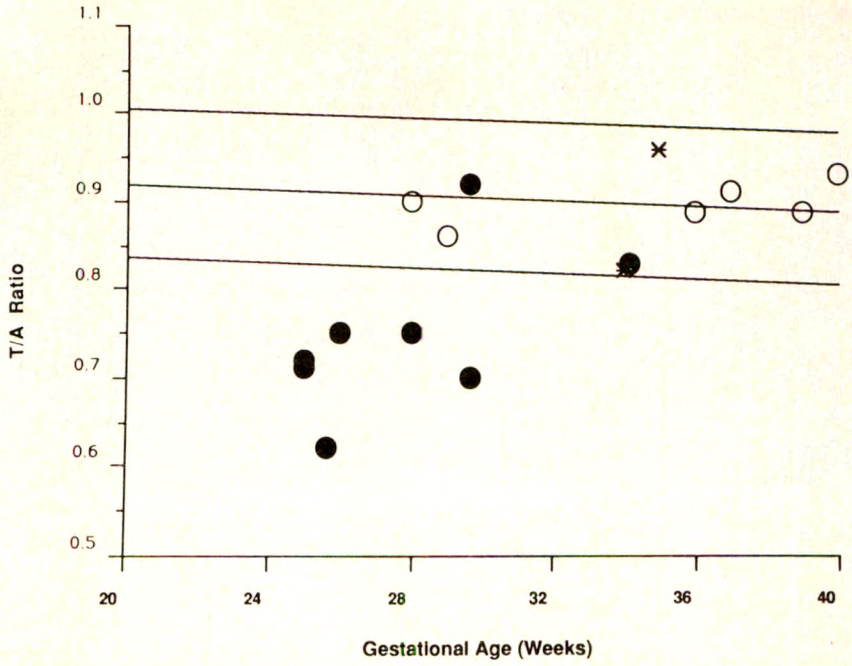


Fig. 2. Final thoracic versus abdominal circumference ratio versus gestational age before delivery for survivors *(open circles)*, survivors with restriction deformities and pulmonary complications *(asterisks)*, and neonatal deaths *(closed circles)*. *Lines* demarcate predicted values ± 2 SDs that are based on 120 women with uncomplicated pregnancies.

racic versus abdominal circumference ratio died from pulmonary hypoplasia determined by autopsy.

Two infants with normal thoracic versus abdominal circumference ratios died early in the neonatal period. The mother of the first experienced premature rupture of fetal membranes at 16.4 weeks' gestation and was delivered of her infant at 29 weeks' gestation. The thoracic versus abdominal circumference ratio on the day of her delivery was 0.92, but the infant died within 20 minutes of birth after failure of ventilation in spite of high ventilation pressures. Autopsy of this infant revealed normal lung weights with the absence of alveolar development.

The second infant, who was delivered at 34 weeks' gestation, died of pulmonary dysplasia after the mother had experienced premature rupture of fetal membranes at 23 weeks' gestation. The thoracic versus abdominal circumference ratio 4 days before delivery was 0.83. This infant died within 5 minutes of birth as a result of failure of ventilation. Lung weights were at the fifth percentile for gestational age. Lung microscopy revealed a typical pattern of pulmonary dysplasia.

Thus the thoracic versus abdominal circumference ratio had a 75% sensitivity and 100% specificity for the prediction of neonatal death from pulmonary disease. The positive and negative predictive values were 100% and 80%, respectively. The sensitivity of the thoracic versus abdominal circumference ratio in the prediction of pulmonary hypoplasia was 100%.

Serial ultrasonographic evaluation revealed appropriate growth of the abdominal circumference in all cases. Thoracic versus abdominal circumference ratios of survivors did not vary significantly among individual assessments and paralleled the predictive curve (Fig. 3, *A*). Seven of eight infants who died from pulmonary disease were followed-up within 1 week of premature rupture of fetal membranes. The eighth woman studied had ruptured membranes at 15 weeks' gestation and was first assessed at 22 weeks' gestation. All seven infants scanned within 1 week of premature rupture of fetal membranes had normal thoracic versus abdominal circumference ratios at the time of first assessment. Thoracic versus abdominal circumference ratios subsequently declined into the abnormal range in five of these fetuses (Fig. 3, *B*).

Eight women demonstrated persistent oligohydramnios to the time of delivery; six of their fetuses had abnormal thoracic versus abdominal circumference ratios. All six of these infants died as a result of pulmonary hypoplasia. Two fetuses whose mothers experienced persistent oligohydramnios had normal thoracic versus abdominal circumference ratios before delivery, and one whose mother had premature rupture of fetal membranes at 17 weeks and delivery of her infant at 35 weeks developed a pneumothorax and demonstrated significant restriction deformities of the lower extremities that required orthopedic splinting. This infant was normal at the 15-month follow-up. The second

infant whose mother had premature rupture of fetal membranes at 23 weeks' gestation and delivery at 34 weeks' gestation as a result of abruptio placentae suffered pneumomediastinum and demonstrated prominent skin folding of the neck that was indicative of intrauterine crowding.

Fetal breathing was seen at each examination in five of the six infants who died of pulmonary hypoplasia. Although fetal breathing movements were not routinely assessed in labor, regular fetal breathing was identified shortly before delivery in both infants who died of pulmonary dysplasia.

Comment

Premature rupture of fetal membranes before 26 weeks' gestation carries the risk of significant maternal morbidity; chorioamnionitis developed in 42% of reviewed mothers at our institution. With a mean latency from premature rupture of fetal membranes to delivery of 61.8 days (range, 31 to 152 days), prolonged maternal hospitalization is common. A decrease in the fetal thoracic circumference has been suggested as a predictor of subsequent neonatal pulmonary hypoplasia subsequent to oligohydramnios.[5, 6]

Callan et al.[7] reported a case of premature rupture of fetal membranes in an infant with pulmonary hypoplasia and a decreased thoracic versus abdominal circumference ratio. They suggested that the decrease in thoracic versus abdominal circumference ratio might be helpful in the antenatal prediction of pulmonary hypoplasia. A subsequent report from this group studied the use of the fetal thoracic versus abdominal circumference ratio in patients with premature rupture of fetal membranes before 28 weeks' gestation and in patients with oligohydramnios and intact membranes.[8] In a comparison of a variety of fetal measurements and ratios in fetuses at risk for pulmonary hypoplasia, Vintzileos et al.[9] found that ultrasonographic assessment was relatively insensitive (33% to 85%) and nonspecific (57% to 85%).

The fetal thoracic versus abdominal circumference ratio remains virtually constant throughout gestation.[9, 10] Chitkara et al.[10] found a mean thoracic versus abdominal circumference ratio of 0.89 with a lower limit of normal of 0.77 (mean, 2 SDs). Vintzileos et al.[9] demonstrated a lower mean thoracic versus abdominal circumference ratio of 0.85. In that study, a thoracic versus abdominal circumference ratio <0.75 was considered normal. Our cross-sectional study confirms these findings, lower limits of normal were 0.83 at 15 weeks and 0.80 at term.

In our study, only patients with premature rupture of fetal membranes before 26 weeks' gestation and without other identifiable causes of oligohydramnios were included. All six patients with pulmonary hypoplasia predicted by abnormal thoracic versus abdominal circumference ratios died of this disease. Two patients with normal thoracic versus abdominal circumference ratios died of pulmonary dysplasia. In both these cases, lung weights were within the normal range, which suggests that prenatal evaluation by thoracic versus abdominal circumference ratio could not have predicted death in these infants. This finding demonstrates that lung size and weight in the normal range do not necessarily predict normal lung function. A normal thoracic versus abdominal circumference ratio in the patient with premature rupture of fetal membranes before 26 weeks can not guarantee a good clinical outcome.

Nimrod et al.[5] have reported that thoracic circumference that is less than the fifth predicted percentile is a predictor of pulmonary hypoplasia. In our patient group, one case of pulmonary hypoplasia predicted accurately with thoracic versus abdominal circumference ratios would have been missed if the thoracic circumference estimation had been used alone, because the infant's overall size was above average. As demonstrated by Vintzileos et al.[9] the use of the abdominal circumference as an internal "control" could help to correct for variation in fetal size at a given gestational age.

The final objective of this study was to assess the use of qualitative assessment of amniotic fluid volume in the prediction of perinatal outcome. All six infants who died of pulmonary hypoplasia had both abnormal thoracic versus abdominal circumference ratios, and their mothers had persistent oligohydramnios. Two infants who died of pulmonary dysplasia and who had normal lung weights had mothers with normal amniotic fluid volumes. The mothers of two surviving infants had persistent oligohydramnios. One of these infants had significant restriction deformities of the extremities, and the other had prominent skin folds of the neck that suggested intrauterine crowding. Both these infants had respiratory complications after delivery: one suffered pneumothorax and the other developed a pneumomediastinum. Perlman et al.[11] have suggested that after 2 weeks of premature rupture of fetal membranes with persistent oligohydramnios, pregnancy should be discontinued because of the high risk of pulmonary hypoplasia. We have found that a poor perinatal outcome is associated with persistent oligohydramnios in women who experience premature rupture of fetal membranes before 26 weeks' gestation. However, two of eight infants (25%) survived and are normal at follow-up.

Ultrasonographic assessment of premature rupture of fetal membranes may be technically difficult as a result of the loss of the fluid-surface interface, juxtapositioning of soft tissue structures, and distortion of

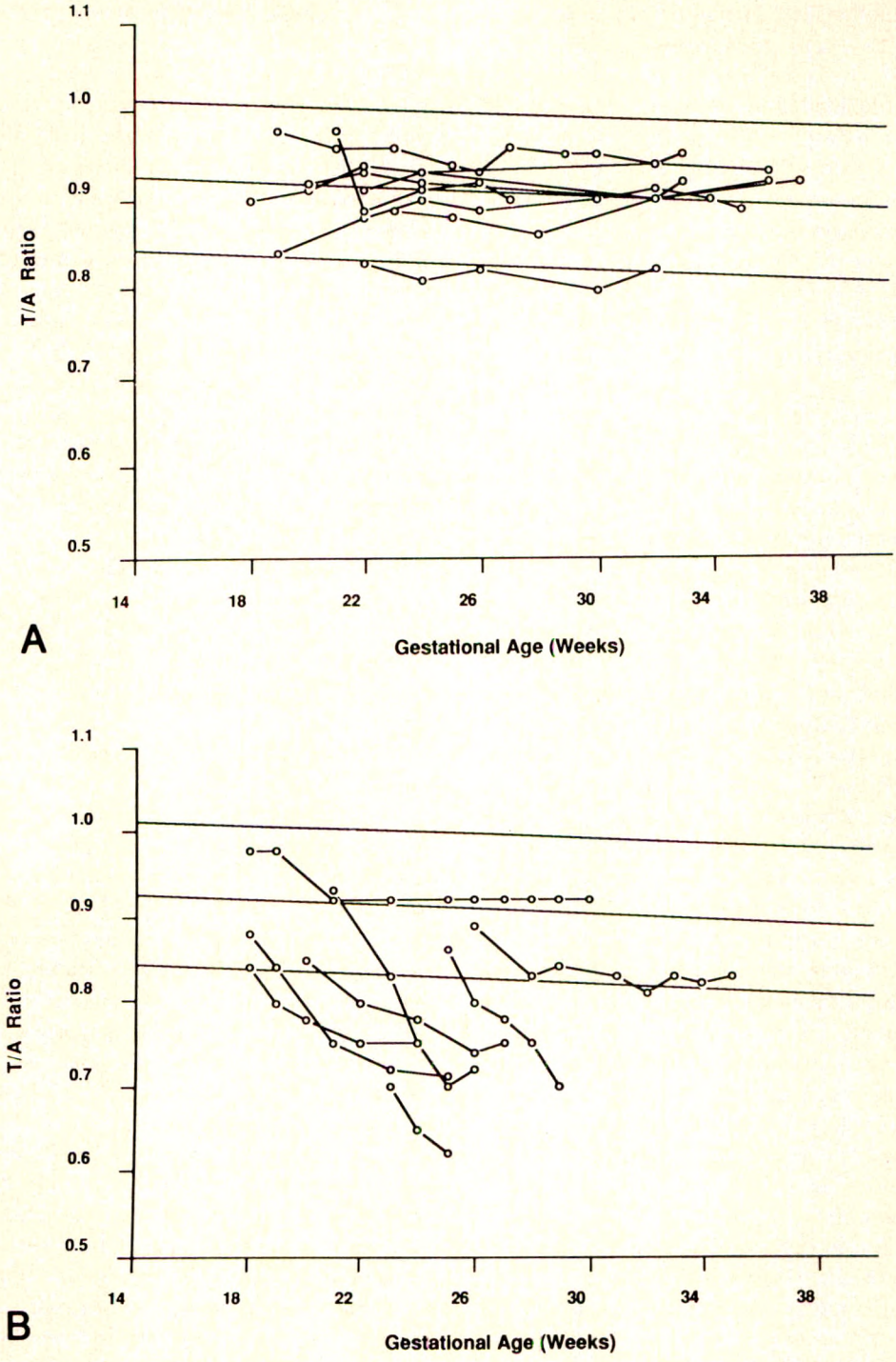


Fig. 3. A, Serial thoracic versus abdominal circumference ratios versus gestational age for surviving infants. **B,** Serial thoracic versus abdominal circumference ratios versus gestational age for nonsurviving infants.

the abdominal circumference. It might be suggested that the chest circumference should be compared with other more consistent internal fetal markers, such as the head circumference or femur length. We have found the thoracic versus abdominal circumference ratio to be reliable and without significant technical difficulty. Any ultrasonographic measurement technique requires the passage of time to identify thoracic growth failure and thus is less sensitive in patients with a short latency. We would expect approximately 5% of the normal population to have measurements smaller than the lower confidence interval for the population (2 SDs below the mean). Serial assessment may be helpful, because these patients should maintain their position on

the standard curve but those infants destined to develop pulmonary hypoplasia demonstrate progressive growth failure (Figs. 3, *A* and 3, *B*). Finally, it is important that patients be compared to a standard curve appropriate to their population. Ideally, normal curves would be constructed at each institution.

The literature contains conflicting results about fetal breathing movements as predictors of lethal pulmonary hypoplasia. In 1987, Blott et al.[12] stated that fetal breathing movements were predictive of a favorable outcome in oligohydramnios. Subsequently, Kilbride et al.[13] and Moessinger et al.[14] disputed this finding. In our evaluation, fetal breathing was consistently identified, regardless of thoracic versus abdominal circumference ratio, in seven of eight infants who subsequently died of pulmonary disease. Thus the presence of fetal breathing movements before labor does not predict good neonatal pulmonary function.

Conclusion

An abnormal thoracic versus abdominal circumference ratio is a reliable predictor of pulmonary hypoplasia. Patients with normal thoracic versus abdominal circumference ratios should be informed that there is still a risk of neonatal death from pulmonary complications. Reaccumulation of amniotic fluid indicates a more favorable prognosis. Patients with persistent oligohydramnios and fetuses with a normal thoracic versus abdominal circumference ratio should be advised of the risk of fetal restriction deformities and the potential for postpartum pulmonary complications. We recommend the use of serial thoracic versus abdominal circumference ratio testing with amniotic fluid assessment in the ongoing clinical management of patients who experience premature rupture of fetal membranes before 26 weeks' gestation.

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Triplet pregnancy involving complete hydatidiform mole and two fetuses: Genetic analysis by deoxyribonucleic acid fingerprint

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A case of a triplet pregnancy involving a dizygous twin pregnancy and a complete hydatidiform mole after therapy with human menopausal gonadotropin and human chorionic gonadotropin is reported. Two female fetuses, two placentas in one mass with two amnions and two chorions, and a tumor mass with a grapelike appearance were spontaneously delivered at 19 weeks of gestation. The deoxyribonucleic acid fingerprints of the two placentas and tumor tissue were compared with those of the parents. The fingerprints of the placentas showed patterns different from each other; however, all their polymorphic fragments could be traced back to either the father or mother. All polymorphic fragments of the tumor tissue were inherited only from the father (androgenesis). These results indicated that this triplet pregnancy involved a dizygous twin pregnancy and a complete hydatidiform mole. (AM J OBSTET GYNECOL 1992;166:664-7.)

Key words: Triplet pregnancy, human menopausal gonadotropin, human chorionic gonadotropin, hydatidiform mole, deoxyribonucleic acid fingerprint

Complete hydatidiform mole is the most common trophoblastic tumor deriving from the fertilization of an anuclear empty ovum by a haploid sperm that duplicates its complementary chromosomes or by two sperms. Although reports vary, it is a well-known fact that the tumor shows specific geographic distribution in its incidence. Recent reports have indicated that the incidence of complete mole is 1 per 1200 pregnancies in the United States, 1 per 500 in Japan, and 1 per 100 in Indonesia.[1-3] However, the incidence of complete hydatidiform mole with a coexisting fetus is not so frequent, being estimated to be 1 per 22,000 to 100,000 pregnancies.[4] There have been only two reports of complete hydatidiform mole in triplet pregnancy with two normal fetuses and placentas.[5, 6] Both cases were pregnancies occurring after ovulation-inducing therapy. Recently, we experienced a case of spontaneous abortion of two living female fetuses and a tumor mass with a grapelike appearance after ovulation-inducing therapy by human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG). To investigate the zygosity of the two fetuses and the pathogenesis of the tumor, we introduced the newly developed method of deoxyribonucleic acid (DNA) fingerprint analysis, which has been reported to be a very useful tool for zygosity determination in multiple pregnancy[7] and the study of pathogenesis of trophoblastic tumors.[8, 9] Comparisons of DNA fingerprints among the fetuses, tumor, and the parents revealed that this case was a triplet pregnancy involving a dizygotic twin pregnancy and a complete androgenic hydatidiform mole. Ovulation induction therapy resulting in the production of immature ova could be one of the risk factors for hydatidiform mole.

Case report

The patient was a 24-year-old woman, gravida 0, para 0. She had received hormonal therapy for anovulation. The pregnancy described here was achieved by hMG and hCG therapy. An ultrasonographic examination at 7 weeks of gestation revealed two living fetuses and two placentas. However, at that time the multiple cystic echo typical of a hydatidiform mole was not identified. At 15 weeks of pregnancy the patient had uterine cramps with a little genital bleeding. She was diagnosed to have threatened abortion and thus was hospitalized. During hospitalization, no clinical symptoms of preeclampsia or hyperthyroidism were recognized, and ultrasonographic examination revealed two living fetuses but did not detect any multiple cystic echo. Twenty-five days later, two female infants, two placentas and a grapelike tumor were delivered. The first fetus was female, weighing 235 gm, and the second fetus was female,

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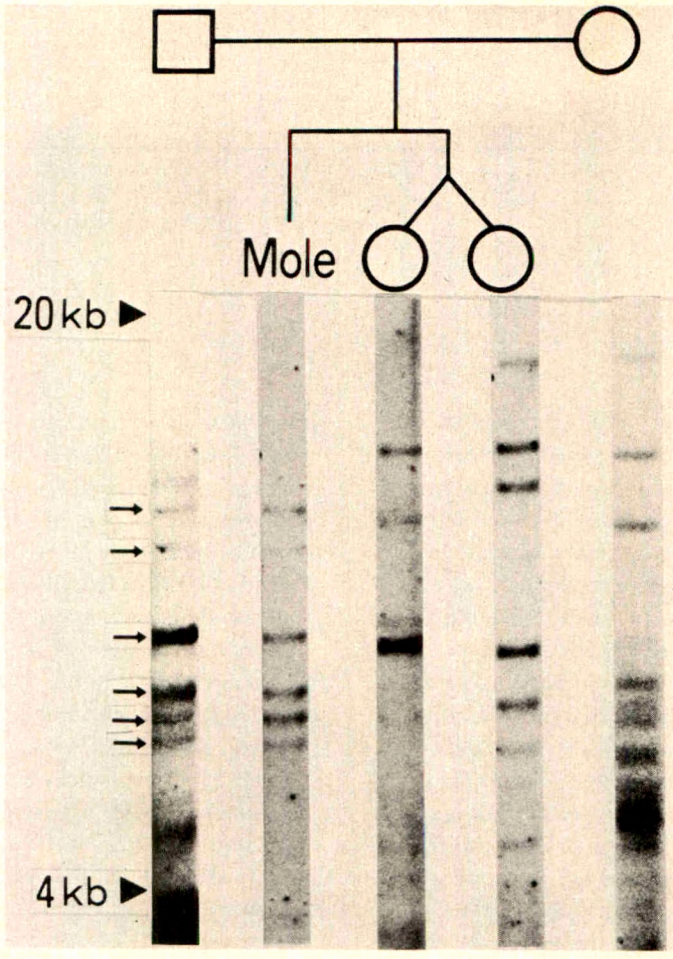


Fig. 1. DNA fingerprints presented by minisatellite DNA probe 33.15 of two placentas, tumor tissue, and parents. All polymorphic fragments of both placentas could be traced back to parents. However, all polymorphism bands in tumor tissues were identified to be of paternal origin *(arrows)*.

weighing 275 gm. They died shortly after birth, and at autopsy they appeared normal. The urinary hCG levels were 110,000 IU/L at delivery, 1100 IU/L at 1 week after spontaneous abortion, and normal at 3 weeks after spontaneous abortion. The urinary hCG level is now being monitored monthly at the clinic. The zygosity of the fetuses and pathogenesis of the aborted tumor were investigated by DNA fingerprint analysis. The high-molecular-weight DNA was extracted from placental tissues, tumor tissue, and peripheral blood of the parents. DNA fingerprint analysis was performed on the extracted DNA with a minisatellite DNA probe 33.15[10] (Fig. 1). The DNA fingerprints of the placentas showed patterns that were different from each other; however, all polymorphic fragments of both placentas could be traced back to the parents, indicating that the fetuses of this pregnancy were dizygotic. All polymorphic fragments of the tumor tissue were inherited only from the father, showing that this tumor was a complete androgenic hydatidiform mole. Histopathologic examination supported this result. These results indicate that the pregnancy described here was a triplet pregnancy involving a dizygotic twin pregnancy and a complete hydatidiform mole.

Comment

There are several possible explanations for the pregnancy containing two living fetuses and a tumor with grapelike appearance: first, the coexistence of a complete hydatidiform mole and a normal twin pregnancy; second, the coexistence of a blighted ovum with diffuse hydropic change and a normal twin pregnancy; third, the coexistence of a partial hydatidiform mole with a living fetus and a normal pregnancy. Clinically, it is very important to discern the multiple pregnancy involving a complete mole from that involving a blighted ovum or partial mole. The relative risks of choriocarcinoma associated with molar pregnancy have been estimated to be 2000 to 4000 times higher than those with normal pregnancy or aborted pregnancy.[11] On the other hand, there has been increasing evidence indicating that partial hydatidiform mole may not be associated with cho-

Table I. Summary of reports on triplet pregnancy consisting of complete hydatidiform mole and twin pregnancy

Report	*Age of patient (yr)*	*Delivery (wk)*	*Fetal weight (gm)*	*Fetal sex*	*Vaginal bleeding*	*Symptoms of preeclampsia*	*Method of differential diagnosis*	*Ovulation induction*
Case 1 (Sauerbrei et al.[5])	23	22	440 370	Male Male	Yes	Yes	Histologic examination	Clomiphene
Case 2 (Ohmichi et al.[6])	34	17	155 166	Female Male	Yes	No	Karyotyping	hMG and hCG
Case 3 (Azuma et al., this report)	24	19	235 275	Female Female	Yes	No	DNA fingerprinting	hMG and hCG

riocarcinoma.[12] In previous studies differential diagnosis of trophoblastic tumors has been performed by histologic examination or by karyotype analysis.[13, 14] However, it is sometimes very difficult to distinguish a blighted ovum with severe hydropic change from a complete mole by histologic examination only, and karyotype analysis requires a time-consuming tissue culture for chromosomal preparation and special techniques for analysis of chromosomal banding patterns.

In this study we used a newly developed method of DNA fingerprint. DNA fingerprints are DNA polymorphisms detected by minisatellite DNA probes that were obtained by Jeffreys et al.[10] from a human genomic DNA library by hybridization screening with a 33 bp tandem repeat probe constructed from myoglobin minisatellite. Minisatellite DNA probes, including probe 33.15, can detect highly variable regions widely dispersed in human genomes and give strictly individual specific DNA polymorphism. This method has been used in the study of the pathogenesis of trophoblastic tumors, and in this case it revealed the zygosity of two fetuses and the pathogenesis of a tumor with grapelike appearance. This method has many other advantages: (1) DNA fingerprinting is a simple and rapid method, and results of genetic analysis of trophoblastic tumors or accurate zygosity determination of multiple pregnancy can be obtained within a few days, (2) DNA fingerprint analysis can be performed not only with fresh materials but also with frozen samples; (3) <0.1 gm of tissue or 0.5 ml of peripheral blood can always provide sufficient DNA for the DNA fingerprint analysis, and the extracted DNA can be preserved at 4° C for an extended time. Therefore this method is a very useful tool for zygosity determination in multiple pregnancy and genetic analysis of trophoblastic tumors. The comparisons of DNA fingerprints among the fetuses, tumor, and their parents revealed that our case was a triplet pregnancy involving a twin pregnancy of dizygosity and a complete androgenic hydatidiform mole. A summary of the results is shown in Table I, together with those of previous studies on triplet pregnancy involving molar pregnancy. As shown in Table I, all the cases of triplet pregnancy involving a complete mole, heretofore reported, are a result of ovulation-inducing therapy by clomiphene or by hMG. It is a well-known fact that clomiphene or hMG sometimes causes ovulation of more than one ovum.

The rate of ovulation of an anuclear "empty" ovum as the underlying cause of complete mole may increase by forcing ovulation of more than one ovum by clomiphene or hMG. As the number of triplet pregnancies involving a complete mole is only three, the relationship between the risks of molar pregnancy and ovulation-inducing therapy is not clear. However, the use of clomiphene and hMG is very popular in therapy for anovulation. The classic features of molar pregnancy, such as uterine enlargement in advanced cases or severe emesis, are sometimes recognized in multiple pregnancy, especially in triplet or quadruplet pregnancy. Therefore it is very important to perform careful examinations to rule out molar pregnancy when patients with multiple pregnancy after ovulation-inducing therapy have genital bleeding. More thorough investigations are required to reveal the relationship between this ovulation-inducing therapy and ovulation of an anuclear "empty" ovum.

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Influence of alcohol intake on postoperative morbidity after hysterectomy

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Postoperative morbidity after hysterectomy was prospectively studied in 229 consecutive patients in our departments. The incidence of alcohol abuse (>60 gm of alcohol daily) and social drinking (between 25 and 60 gm of alcohol daily) was 6.5% for each. When compared with the social drinkers and the control group, the alcohol abuse group had significantly more complications (80% vs 27% and 80% vs 13%, respectively). (Am J Obstet Gynecol 1992;166:667-70.)

Key words: Postoperative morbidity, alcohol abuse, hysterectomy

Recent studies have retrospectively shown that male alcoholics develop significantly more complications after both major and minor, elective and emergency surgery.[1-3] However, the knowledge of outcome after operation in alcoholic women is sparse. Therefore the aim of this study was to investigate the influence of alcohol intake on postoperative morbidity in women after hysterectomy.

Material and methods

This prospective study included 229 consecutive patients who underwent elective hysterectomy (from Nov. 1, 1988, to Oct. 31, 1989) at two Copenhagen centers. The indications were benign or malignant tumor or adenomyosis and endometriosis. Patients undergoing acute subtotal or vaginal hysterectomy or hysterectomy combined with colporrhaphy were not included. The patients were interviewed preoperatively on present and previous smoking habits and daily alcohol consumption. According to the actual consumption (i.e., up to the admission for hysterectomy) the patients were divided into three groups: light drinkers who drank <25 gm (fewer than two Danish drinks) of alcohol daily, moderate drinkers who consumed between 25 and 60 gm of alcohol a day, and patients who drank ≥60 gm (five or more Danish drinks) daily and were defined as heavy drinkers (one Danish drink contains 12 gm pure alcohol).

Cardiopulmonary and endocrine diseases requiring treatment were recorded, together with gynecologic symptoms and diagnoses, the surgical procedure and its duration, and blood loss and transfusion. Finally, morbidity that developed within the first 14 postoperative days was noted. Complications were defined by clinical symptoms and the need for treatment, i.e., septicemia with a generally septic condition and bacteriemia, bleeding episodes necessitating blood transfusion.

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Table I. Characteristics of heavy, moderate, and light drinkers

	Heavy (n = 15)	*Moderate (n = 15)*	*Light (n = 199)*
Age (yr, median and range)	45 (30-72)	45 (30-69)	47 (22-86)
Weight (kg, median and range)	69 (53-106)	63 (50-84)	63 (43-108)
Height (cm, median and range)	163 (153-169)	165 (158-178)	165 (150-180)
Smokers (gm/day, median and range)	20 (10-30)	15 (4-20)	15 (3-25)
Nonsmokers (%)	7*	27	51
Condition requiring treatment			
Heart failure (%)	0	7	6
Angina pectoris (%)	0	0	1
Hypertension (%)	0	0	3
Asthma (%)	0	0	2
Chronic bronchitis (%)	20†	0	3
Insulin-dependent diabetes mellitus (%)	0	0	2
Non-insulin-dependent diabetes mellitus (%)	0	0	2
Specific symptom			
Metrorrhagia (%)	80	67	66
Dysmenorrhea (%)	13	7	10
Chronic pelvic pain (%)	27	33	39
Dyspareunia (%)	7	13	7
Uterine disease			
Fibroid (%)	33	67‡	39§
Carcinoma in situ (%)	0	0	3
Cervical carcinoma, stage I (%)	13	0	1
Adenomatous hyperplasia (%)	0	0	4
Corpus carcinoma (%)	14	13	19
Adenomyosis (%)	0	7	8
Ovarian disease			
Cyst (%)	13	7	6‖
Fibroma (%)	0	0	1
Carcinoma in situ (%)	0	0	3
Carcinoma (%)	14	0	8
Endometriosis (%)	0	7	3
Normal histologic results (%)	13	0	5
Surgical procedure			
Hysterectomy only (%)	40	47	38
Hysterectomy with partial adnexal resection (%)	13	7	5
Hysterectomy with salpingo-oophorectomy (%)	47	46	57
Hysterectomy with supplemental omentectomy (%)	7	7	15
Duration of operation (min, median and range)	85 (60-165)	90 (45-140)	90 (45-190)
Blood loss (L, median and range)	0.3 (0.1-2)	0.3 (0.1-1)	0.3 (0.1-2)
Blood transfusion (L, median and range)	0 (0-1.4)	0 (0-0.9)	0 (0-1.2)

*$p \leq 0.001$ compared with light drinkers, χ^2 test.
†$p \leq 0.01$ compared with light drinkers.
‡$p \leq 0.05$ compared with light drinkers.
§An additional 6% had malignant diagnosis.
‖An additional 7% had malignant diagnosis.

pneumonia diagnosed by stethoscope or chest roentgenogram and treated with antibiotics, and wound infection requiring intervention. Age, weight, and height were noted.

The χ^2 test and the Mann-Whitney test were used in the analysis of unpaired data. The level of significance was $p < 0.05$.

Results

The prevalences of heavy and moderate drinkers were similar, 6.5%. The three groups were comparable regarding preoperative characteristics and symptoms and diagnoses, except for fewer nonsmokers (7% vs 27% and 51%) and higher incidence of chronic bronchitis (20% vs 0% and 2%) in the heavy drinkers and more uterine fibroids in the moderate drinkers compared with the light drinkers (67% vs 39%) (Table I). There were no differences among the groups with regard to surgical procedure, duration of surgery, or perioperative blood loss and transfusion (Table I).

The heavy drinkers had significantly higher postoperative morbidity, compared with the moderate drinkers (80% vs 27%, $p \leq 0.01$, χ^2 test) and the light drinkers (80% vs 13%, $p \leq 0.01$) (Table II). There was no difference in postoperative morbidity between the moderate and light drinkers.

Regional infections were the most frequent complications seen in all three groups. Wound infection, cys-

Table II. Postoperative morbidity in heavy, moderate, and light drinkers after hysterectomy

	Heavy (n = 15)	*Moderate (n = 15)*	*Light (n = 199)*
Minor complication			
Wound rupture (%)	13	0	1
Wound infection (%)	47*	13	4
Pneumonia (%)	13	0	2
Vaginal abscess (%)	7	7	0
Cystitis (%)	13	0	9
Adynamic ileus (%)	7	0	1
Major complication (%)			
Fascia rupture (%)	7	0	1
Bleeding (%)	13	13	2
Intraabdominal abscess (%)	7	0	0
Pulmonary embolism (%)	0	0	1
Delirium or psychosis (%)	7	0	0
Halothane hepatitis (%)	0	0	1
Patients with complications (%)	80*	27	13
Hospital stay (days, median and range)	14 (7-27)	7 (4-18)	7 (3-31)

*$p \leq 0.001$, χ^2, when compared with the control group.

titis, pneumonia, and vaginal abscess developed in 67% of the heavy drinkers, 20% of the moderate drinkers ($p \leq 0.01$), and 10% of the light drinkers ($p \leq 0.001$). However, no patient had generalized infection, and there was no perioperative mortality. The heavy drinkers had significantly prolonged hospital stays (Table II).

Finally, the light drinkers were divided according to smoking habits, but there was no difference in postoperative morbidity between smokers and nonsmokers (15% vs 11%).

Comment

Our study clearly demonstrates that the overall complication rate is heightened in female patients who are heavy drinkers (i.e., ≥60 gm of alcohol a day). Smoking may also be a part of the explanation for the increased postoperative morbidity of the alcohol-drinking group because of fewer nonsmokers in this group, corresponding to the association between smoking and alcohol abuse.[1] The heavy drinkers suffered more often from chronic bronchitis. However, the incidence of respiratory complications and cough-induced wound rupture was not significantly increased in this group. The high complication rate among the heavy drinkers is in accordance with recent studies on alcoholic men undergoing surgery.[2-4] In these retrospective materials the alcoholic groups (consuming ≥60 gm alcohol daily) were matched to control patients (drinking <25 gm alcohol daily) regarding smoking habits, thus minimizing a possible influence of tobacco on complications after surgery. Furthermore, smoking did not influence the development of complications after surgery in the control group. We therefore speculate that alcohol intake, compared with smoking, might be a dominant factor in the etiology of postoperative morbidity.

The most frequent complication was wound infection, possibly because of the fact that alcohol interferes with normal immune host defense mechanism.[5]

Alcohol also has an inhibitory effect on hemostatic function,[6] but we failed to demonstrate more bleeding episodes among the heavy drinkers.

The postoperative morbidity of our light drinkers corresponds to the incidence of complications in the control groups from other studies, except for simple pyrexia or febrile morbidity, which we did not measure as a specific complication.[7-10] Also the hospital stay after surgery in the light drinkers is in agreement with that for control patients in other investigations.[8, 11] However, in these studies a history of alcohol habits was not mentioned.

There are few reports of alcohol consumption in women with gynecologic diseases. Halliday et al.[12] found that alcohol abuse is common in women seeking gynecologic care, and Moore et al.[13] found that 12.5% of patients in gynecologic and obstetric departments were alcohol abusers, drinking >50 drinks a week. We found only a 6.5% incidence of heavy drinkers and 6.5% of moderate drinkers, but our study group was older and admitted for elective operation, which may influence the incidence of alcohol abuse.

Studies concerning alcohol intake are often defective because of underestimated self-reports of consumption. Especially among heavy drinkers there is a tendency to minimize abuse.[14] Because alcohol consumption is underestimated in self-reports, one might expect that some patients from the light drinkers, in fact, belong to the heavy drinking group, whereas the reverse situation seems unlikely. Since the study showed remarkable differences in morbidity between the light and heavy drinkers, the problem of uncertain self-reports should not change the conclusion.

The presented data call for more extensive preoperative evaluation concerning drinking habits to point out a group at special risk during surgery.

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Effects of β-hydroxybutyrate infusion on hind limb metabolism in fetal sheep

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OBJECTIVES: This study investigated the effects of high ketone levels on fetal carcass metabolism. Specifically, we aimed to determine whether β-hydroxybutyrate was taken up by fetal muscle and whether lactate production by the fetal hind limb contributed to the raised circulating lactate levels seen in high ketone states.
STUDY DESIGN: Hind limb metabolism was studied in 10 chronically catheterized fetal sheep. Substrate/oxygen quotients were measured before and after 2 hour infusions of β-hydroxybutyrate and compared with the Wilcoxon signed-rank test.
RESULTS: β-Hydroxybutyrate was taken up by hind limb tissues in large amounts (butyrate/oxygen quotient = 1.02 ± 0.40). Lactate was produced by the hind limb (lactate/oxygen quotient = −0.96 ± 0.52) in amounts almost equivalent to glucose uptake (glucose/oxygen quotient = 1.32 ± 0.29). Hind limb oxygen consumption increased 35% and fetal arterial oxygen content fell 16%.
CONCLUSION: β-Hydroxybutyrate may be consumed by the fetal carcass in amounts sufficient to entirely substitute for glucose. Glucose may then be released as lactate for metabolism elsewhere. Ketones may be important fetal substrates during maternal starvation. (Am J Obstet Gynecol 1992;166:671-6.)

Key words: Ketones, fetal carbohydrate metabolism, lactate, fetal sheep

The ketones, β-hydroxybutyrate and acetoacetate, provide important alternatives to glucose as metabolic substrates during starvation in many mammalian species. In pregnancy, maternal ketosis occurs frequently, and ketones cross the placenta in a number of species, including human, to form potential alternative substrates for fetal metabolism.[1] Ketones are readily taken up by several fetal tissues, including muscle and liver,[1, 2] and may be an important substrate for fetal brain metabolism when glucose concentrations are low.[3]

However, in humans maternal ketosis is associated with poor fetal outcome.[4, 5] Studies in sheep have shown that direct infusion of β-hydroxybutyrate to the ewe or fetus may be associated with reduced fetal oxygenation and consistently results in a rapid and prolonged rise in circulating lactate concentrations.[3, 6-9] The reason for this rise in lactate is unknown. Lactate is an important fetal substrate under normal circumstances and is both consumed from the placenta and produced by fetal tissues in large amounts.[10-12] The magnitude of the change in fetal lactate concentrations during ketone infusion suggests that carbohydrate metabolism must be affected in major fetal organs. The most likely possibilities include inhibition of lactate uptake in the fetal liver or increased lactate production in fetal placenta or muscle. Since the carcass (mainly muscle) constitutes approximately 70% of body weight in fetal sheep,[13, 14] we chose to explore this last possibility by studying the effects of ketone infusion on hind limb metabolism in fetal sheep. We aimed to determine whether β-hydroxybutyrate was taken up by fetal muscle and whether lactate production by the fetal hind limb contributed to the raised circulating lactate levels seen during these infusions.

Material and methods

Animal preparation. Ten pregnant ewes were surgically prepared while they were under either general anesthesia (althesan induction, 2% halothane in oxygen maintenance) or spinal anesthesia (1% tetracaine hydrochloride with intravenous pentobarbital sodium and ketamine hydrochloride sedation as required). Polyvinyl catheters were placed in a maternal femoral artery and vein or carotid artery and jugular vein. Hysterotomy was performed through a midline abdominal incision, and polyvinyl catheters were placed in a fetal femoral artery and vein through the tarsal artery and vein using 1% xylocaine for local anesthesia. An ad-

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ditional infusion catheter was placed in another fetal vein, usually the brachial. The uterine and maternal abdominal incisions were then closed.

Ewes were allowed to recover for at least 5 days after surgery. They were housed individually with free access to food and water. Catheters were flushed daily with heparinized saline solution. Antibiotics (penicillin and gentamicin) were given daily to the ewe and fetus for 3 to 5 days after surgery.

At the end of all studies the ewes were killed with an overdose of thiopental sodium. The position of the catheters was carefully checked to ensure that the fetal femoral catheters lay in either the femoral or internal iliac vessels. Thus the blood sampled represented that supplying and draining only the hind limb. The fetus was then weighed and dissected. The catheterized hind limb was removed by dividing the tissues at the level of the femoral head and placed in formalin.

All aspects of the experimental protocols and animal handling were approved by the Animal Ethics Committee of the institutions in which the experiments were performed.

Experimental protocols. Eleven experiments were performed in 10 animals at 121 to 139 days' gestation (130 ± 2.1 days, mean ± SEM). Each experiment consisted of a 2-hour infusion of sodium β-hydroxybutyrate, followed by a 2-hour recovery period. Sodium β-hydroxybutyrate was made up in distilled water, neutralized to pH 7.4, and infused through a 0.45 μm filter through the fetal venous catheter after an initial 1 ml bolus. The concentration was calculated to give 0.1 mmol/kg of estimated fetal weight per minute when infused at 0.1 ml/min.

Blood samples were taken immediately before and at hourly intervals during the 4-hour study period from the maternal artery and fetal femoral artery and femoral vein. In five animals, radiolabeled microspheres (15 μm, Minnesota Mining & Manufacturing, St. Paul, Minn.) were injected through the femoral venous catheter before and after the 2-hour infusion period for measurement of blood flow. The reference sample was drawn from the femoral artery.

Samples. Samples for blood gas analysis (0.4 ml) were drawn into 1-ml heparinized syringes on ice and immediately analyzed in duplicate with a Corning 158 (Corning Diagnostic, Medfield, Mass.) or Radiometer ABL 330 blood gas analyzer and a Radiometer OSM2 hemoximeter (Radiometer, Copenhagen). Blood samples for metabolite assays (1.4 ml) were drawn into heparinized syringes and immediately deproteinized on ice. Samples were then centrifuged at 4° C and the supernatant decanted and frozen at −70° C until assay.

All assays were performed in duplicate, and all samples from one experiment were assayed in the same batch. Glucose was measured with a glucose oxidase method, and lactate and pyruvate were measured with standard enzymatic spectrophotometric techniques (Sigma Chemical Co., St. Louis). β-Hydroxybutyrate was determined by the iron-chelate method.[15] The mean percentage errors between duplicate samples were glucose 3.0, lactate 2.5, pyruvate 10.7, and β-hydroxybutyrate 3.7.

For measurement of hind limb blood flow, tissues were carbonized at 325° C for at least 24 hours, powdered, and packed into vials for counting on an Ultima II Multichannel Pulse Height Analyser (Ino Tech, Co., Fort Atkinson, Wis.). Isotopes used included chromium 51, strontium 85, niobium 95, manganese 54, zinc 65, gadolinium 153, and indium 114. Blood flow to the hind limb was calculated from counts in the reference sample.[16, 17]

Calculations. Blood oxygen contents were calculated from measured P_{O_2}, hemoglobin, and oxygen saturations. Metabolite concentrations were all expressed as the mean of duplicate samples. Hind limb substrate/oxygen quotients were calculated as the arteriovenous difference for the substrate across the hind limb × K divided by the arteriovenous difference for oxygen, where K = 6 for glucose, 3 for lactate and pyruvate, and 4.5 for β-hydroxybutyrate.

All results for the two experiments performed on one of the animals were averaged, and this single average value was used in statistical analyses. Hind limb substrate uptakes were calculated as the arteriovenous substrate difference × the blood flow per 100 gm of tissue.

Preinfusion values were compared with values obtained during and after infusions using the Wilcoxon signed-rank test for paired data. All values are expressed as the mean ± standard error of the mean.

Results

Infusion of β-hydroxybutyrate into the fetus resulted in a 54-fold rise in arterial β-hydroxybutyrate concentrations (0.09 ± 0.01 at 0 hour to 5.41 ± 0.88 mmol at 2 hours, $p < 0.001$), which fell during the 2-hour recovery period (0.92 ± 0.31 mmol at 4 hours). Lactate and pyruvate concentrations both rose more than twofold during the infusion and continued to rise during the recovery period (Table I, Fig. 1). Fetal arterial concentrations of glucose and oxygen were unchanged during the infusion, but there was a 14% rise in glucose concentration and a 16% fall in oxygen content by the end of the recovery period (Table I, Fig. 1). There were no changes in fetal pH (7.35 ± 0.01) or blood gases (P_{O_2} = 20.0 ± 1.3) at any time during the studies and no change in any of the maternal blood gas and metabolite concentrations measured (maternal pH = 7.46 ± 0.01, P_{O_2} = 109.5 ± 4.3 torr, β-hydroxybutyrate = 1.08 ± 0.23 mmol/L, glucose =

Table I. Fetal hind limb metabolic data before, during, and after β-hydroxybutyrate infusion

	Arterial concentration (mmol/L)	*Arteriovenous difference (mmol/L)*	*Substrate/oxygen quotient*	*Substrate uptake (μmol/min/100 gm)*
β-Hydroxybutyrate				
0 hour	0.09 ± 0.01	0.01 ± 0.01	0.06 ± 0.03	0.2 ± 0.2
2 hours	5.41 ± 0.88*	0.21 ± 0.08*	1.02 ± 0.40†	7.3 ± 3.5‡
4 hours	0.92 ± 0.31*	0.01 ± 0.01	0.03 ± 0.05	
Glucose				
0 hour	1.02 ± 0.13	0.17 ± 0.03	1.32 ± 0.29	8.1 ± 1.7
2 hours	1.10 ± 0.12	0.16 ± 0.02	1.29 ± 0.18	4.7 ± 0.9
4 hours	1.16 ± 0.14*	0.18 ± 0.03	0.95 ± 0.51	
Lactate				
0 hour	1.51 ± 0.11	−0.00 ± 0.03	0.05 ± 0.10	−2.6 ± 2.0
2 hours	3.64 ± 0.33*	−0.21 ± 0.08†	−0.96 ± 0.52*	−6.6 ± 2.7†
4 hours	3.73 ± 0.60*	−0.14 ± 0.07†	0.23 ± 0.61	
Pyruvate				
0 hour	0.05 ± 0.01	0.01 ± 0.01	0.04 ± 0.03	0.1 ± 0.1
2 hours	0.12 ± 0.01†	0.00 ± 0.01	0.01 ± 0.03	−0.3 ± 0.3
4 hours	0.14 ± 0.03†	0.00 ± 0.01	0.01 ± 0.03	
Oxygen				
0 hour	2.58 ± 0.22	0.83 ± 0.06	—	24.8 ± 2.9
2 hours	2.33 ± 0.23	0.82 ± 0.07	—	33.6 ± 4.5‡
4 hours	2.18 ± 0.23*	0.67 ± 0.12	—	

Values are mean ± SEM for 10 studies in the first three columns and five studies in the last (substrate uptake) column.
*$p < 0.01$ for comparison with value before beginning infusion (0 hour).
†$p < 0.05$ for comparison with value before beginning infusion (0 hour).
‡$p = 0.067$ for comparison with value before beginning infusion (0 hour).

3.03 ± 0.24 mmol/L, lactate = 0.81 ± 0.09 mmol/L, pyruvate = 0.04 ± 0.01 mmol/L).

β-Hydroxybutyrate infusion resulted in a significant arteriovenous difference for β-hydroxybutyrate across the hind limb, so that by the end of the infusion β-hydroxybutyrate uptake could have accounted for all oxidative requirements of the hind limb (butyrate/oxygen quotient at 2 hours = 1.02 ± 0.40). However, this uptake was closely matched by an increased output of lactate by the hind limb (lactate/oxygen quotient at 2 hours = −0.96 ± 0.52). The glucose/oxygen quotient across the hind limb (1.32 ± 0.29) was unchanged throughout the study. There was no significant arteriovenous difference for pyruvate at any time across the hind limb, and no significant change in the arteriovenous difference for oxygen (Table I). Thus the total calculated substrate/oxygen quotient across the hind limb, the sum of the quotients for β-hydroxybutyrate, lactate, glucose and pyruvate, was unchanged throughout the study (1.52 ± 0.27 at 0 hour, 1.36 ± 0.98 at 2 hours, and 1.26 ± 0.47 at 4 hours, Fig. 2).

The mean blood flow to the hind limb for the five animals in which it was measured was 38.9 ± 5.4 ml/min/100 gm and did not change after β-hydroxybutyrate infusion (36.7 ± 6.5 ml/min/100 gm at 2 hours). β-Hydroxybutyrate uptake across the hind limb reached 7.3 ± 3.5 μmol/min/100 gm by the end of the infusion, a figure almost exactly matched by the simultaneous hind limb output of lactate (6.6 ± 2.7 μmol/min/100 gm) and greater than the simultaneous uptake of glucose (4.7 ± 0.9 μmol/min/100 gm). There was no significant uptake of pyruvate. Hind limb oxygen uptake increased by 35% by the end of the infusion (Table I).

Comment

This study has shown that β-hydroxybutyrate may be taken up in large amounts by the tissues of the fetal hind limb. In spite of this large uptake of an alternative substrate, the total hind limb substrate/oxygen quotient was unchanged. β-Hydroxybutyrate uptake was matched by a substantial production of lactate, and there was no change in hind limb glucose uptake. Because the coefficients of extraction for the measured substrates were low across the hind limb, the methodology used in this study may allow substantial errors in the calculation of metabolic balances such as are attempted here. Radiolabeled tracer turnover studies are required to confirm our observations. Nevertheless, the data are consistent with other published studies and with what is known about the metabolic pathways involved (see below).

The lactate production by the hind limb is likely to have been from carbon originating in glucose rather than β-hydroxybutyrate. β-Hydroxybutyrate is metabolized to acetyl-coenzyme A, which in mammalian tissues cannot be converted to pyruvate and hence lactate.[18] On the other hand, glucose is normally metabolized to pyruvate before entry to the citric acid cycle.

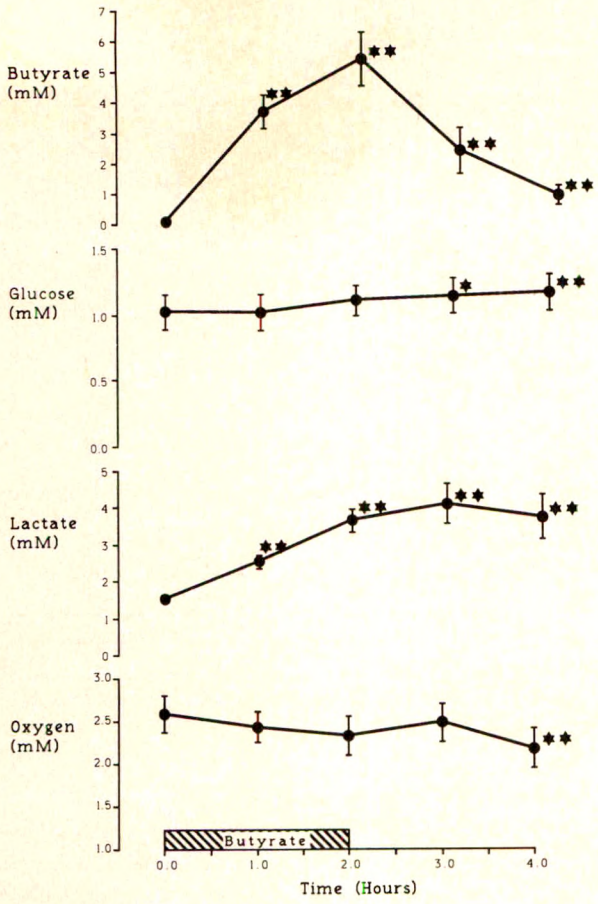


Fig. 1. Changes in fetal arterial substrate and oxygen concentrations during and after β-hydroxybutyrate infusion. Figures are mean ± SEM for 10 studies. β-Hydroxybutyrate was infused during the first 2 hours *(hatched bar)*. *Asterisk*, $p < 0.05$; *double asterisk*, $p < 0.01$ for comparison with value before beginning infusion (0 hour).

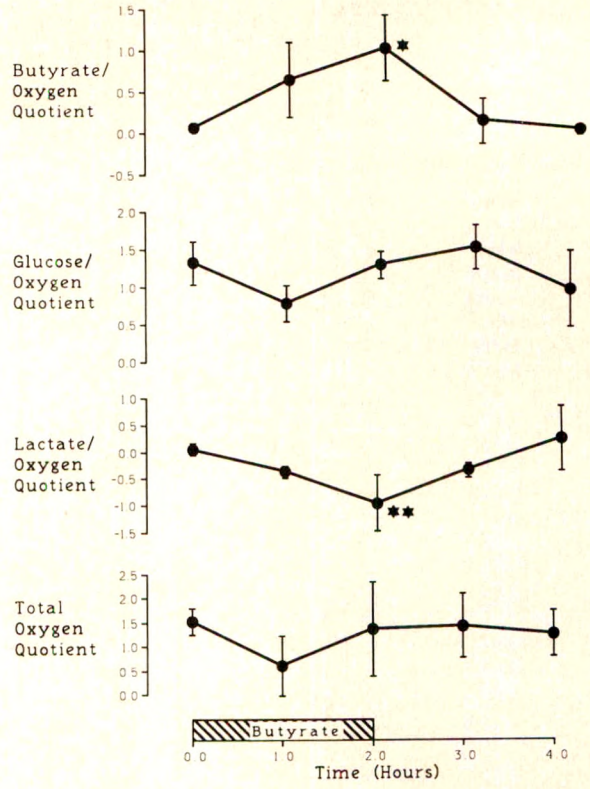


Fig. 2. Changes in fetal hind limb substrate/oxygen quotients during and after β-hydroxybutyrate infusion. Figures are mean ± SEM for 10 studies. β-Hydroxybutyrate was infused during the first 2 hours *(hatched bar)*. *Asterisk*, $p < 0.05$; *double asterisk*, $p < 0.01$ for comparison with value before beginning infusion (0 hours).

In the presence of β-hydroxybutyrate, this pyruvate may instead be converted to lactate due to inhibition of pyruvate dehydrogenase by accumulating acetyl-coenzyme A and the reduced form of α-nicotinamide adenine dinucleotide.[1, 18] Thus it is likely that during β-hydroxybutyrate infusion, glucose is metabolized to pyruvate and then to lactate by the hind limb tissues, whereas β-hydroxybutyrate, through acetyl-coenzyme A, enters the citric acid cycle, substituting for glucose in providing unchanged total substrate supplies for oxidative metabolism. This mechanism would provide an efficient pathway by which, when ketones are available, they could be used as alternative substrates by fetal muscle. Glucose carbon could then be released as lactate for uptake by other tissues, maintaining carbohydrate in the fetal circulation because lactate does not cross the placenta easily. This released lactate may substitute for glucose as a substrate in other fetal tissues, such as liver[19] or heart,[20, 21] and thus reduced tissue use of glucose may account for the observed rise in circulating fetal glucose concentrations during these studies.

An estimate of the magnitude of this substitution of substrates can be obtained from these studies. Assuming hind limb tissues are representative of whole carcass, which forms approximately 70% of body weight in fetal sheep,[13, 14] then the estimated carcass uptake of glucose in these studies (30 to 50 μmol/min/kg) is in the range of estimated whole body uptake of glucose by the fetal sheep across the placenta.[22-24] This glucose then appears to be converted by the carcass to lactate in amounts (46 μmol/min/kg) that are also similar to the total lactate uptake from the placenta.[10-12] Carcass uptake of β-hydroxybutyrate (51 μmol/min/kg) accounts for approximately half of the infused butyrate (100 μmol/min/kg), suggesting that other tissues in either the fetus or placenta may be taking up butyrate. There is also accumulation of butyrate in the circulation and probably some loss across the placenta and metabolism in maternal tissues. Thus provision of β-hydroxybutyrate to the fetus may enable the carcass to substitute butyrate for a major proportion of its glucose

uptake and allow lactate release in amounts similar to those normally acquired by the fetus from the placenta.

In this study the large lactate release from fetal tissues was not accompanied by any change in fetal pH and resulted in a modest (16%) fall in fetal arterial oxygen content. This is in contrast with previous studies in which β-hydroxybutyrate infusion into the pregnant ewe or fetus resulted in much larger falls in fetal Po_2.[7-9] There are a number of possible reasons for this difference. Miodovnik et al.[7-9] infused larger doses of β-hydroxybutyrate in their experiments and may have observed larger changes in fetal oxygenation for this reason. They also gave simultaneous infusions of antipyrine. Because both β-hydroxybutyrate[7] and antipyrine infusions[25] may be associated with reduced placental blood flow, the combination may have contributed to the observed fall in fetal Po_2. Those studies also resulted in larger rises in fetal blood glucose concentrations than we observed. It is not clear whether this higher glucose was the result of the higher β-hydroxybutyrate dose infused and may itself have contributed to the greater fall in fetal Po_2 or whether the observed greater fetal hypoxemia contributed to the greater rise in blood glucose levels.[7, 8] Whatever the mechanism, it appears that fetal β-hydroxybutyrate infusion is associated with increased hind limb oxygen consumption and impaired fetal oxygenation in what may be a dose-dependent fashion. This may contribute to the poor perinatal outcome in the diabetic pregnancy.

The mean glucose/oxygen quotient measured across the hind limb in these studies was 1.32, suggesting that glucose was taken up in excess of hind limb oxygen requirements. This is consistent with previous studies in fetal sheep, showing that the glucose/oxygen quotient across the hind limb is proportional to the fetal arterial glucose concentration.[14, 26] Indeed, our results are remarkably consistent with the regression equation calculated by Morriss et al.[26]; using their equation, the expected hind limb glucose/oxygen quotient for our data would be 1.43. The fate of the glucose which is taken up in excess of oxidative requirements is not known, but it may perhaps be stored as glycogen or fat to meet later postnatal carbohydrate needs.

In summary, we have shown that β-hydroxybutyrate is taken up in large quantities by the tissues of the fetal hind limb. It appears to almost completely substitute for glucose as an oxidative fuel, resulting in lactate release. This is associated with increased hind limb oxygen consumption and reduced fetal arterial oxygen content. Ketones may be important fetal substrates during times of inadequate glucose supply, such as during maternal starvation.

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Regulation of growth of normal ovarian epithelial cells and ovarian cancer cell lines by transforming growth factor–β

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OBJECTIVE: The purpose of this study was to study the role of transforming growth factor–β in regulation of proliferation of normal and malignant ovarian epithelial cells.
STUDY DESIGN: We examined production of and responsiveness to transforming growth factor–β in primary monolayer cultures of epithelial cells from five normal human ovaries and in five ovarian cancer cell lines.
RESULTS: In normal ovarian epithelial cells, proliferation always was inhibited by transforming growth factor–β (>40%) ($p < 0.01$). Among the cancer cell lines, proliferation of one was markedly inhibited (>95%) ($p < 0.01$), two were only modestly inhibited (15% to 20%) ($p < 0.05$), and two were unaffected. In addition, we found that all of the normal ovarian epithelial cells and four of five ovarian cancer cell lines produce transforming growth factor–β ribonucleic acid and protein.
CONCLUSIONS: These data suggest that transforming growth factor–β may act as an autocrine growth inhibitory factor for normal ovarian epithelium in vivo. Because most of the ovarian cancer cell lines are relatively resistant to the growth inhibitory effect of transforming growth factor–β and because one cell line does not produce transforming growth factor–β, it is possible that loss of the transforming growth factor–β pathway may play a role in the development of some ovarian cancers. (Am J Obstet Gynecol 1992;166:676-84.)

Key words: Ovarian cancer, growth inhibitor, transforming growth factor-β

Transforming growth factor–β (TGF-β) initially was discovered in the culture media of virally transformed cells. It was called a transforming growth factor due to its ability to act in concert with TGF-α to elicit malignant transformation of some normal nontransformed cells. Subsequently, however, it has been demonstrated that TGF-β also is produced by a wide range of normal cells. Molecular cloning of TGF-β has revealed three closely related forms (TGF-β_1, TGF-β_2, TGF-β_3), all of which are composed of two amino acid chains held together by disulfide bonds. In addition, it has been shown that TGF-β is secreted in an inactive form noncovalently bound to a portion of its precursor, from which it must be released to produce biologically active TGF-β.[2]

Although TGF-β initially was discovered due to its ability to induce the transformed phenotype in some normal cells, the predominant effect of TGF-β on most normal cells in vitro is inhibition of proliferation. It has been proposed that TGF-β may also act as a growth inhibitory factor in vivo; however, the sites of origin and physiologic role of TGF-β in various organ systems is not yet entirely clear. In addition, it has been proposed that the unrestrained proliferation of some cancers may, in part, be due to loss of normal growth

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inhibitory pathways such as TGF-β.[2] This hypothesis also needs to be tested rigorously.

In the rodent ovary, it has been shown that both granulosa and theca cells produce TGF-β and that TGF-β regulates both proliferation and responsiveness to gonadotropins of granulosa cells.[3-6] Production of and responsiveness to TGF-β in ovarian epithelial cells, from which the majority of ovarian cancers in the United States arise, has not previously been studied, however. In addition, although it is appreciated that epithelial ovarian cancers spread intraperitoneally due to implantation and growth of cells exfoliated by the primary ovarian neoplasm, the factors that regulate the growth of ovarian cancers remain poorly understood. We have performed a series of experiments designed to investigate the role of TGF-β in growth regulation and transformation of human ovarian epithelium.

Material and methods

Cell culture. Five epithelial ovarian cancer cell lines previously established in our lab (OVCA 420, OVCA 429, OVCA 432, OVCA 433, DOV 13) were routinely cultured in modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum. Primary monolayer cultures of normal human ovarian epithelial cells from five patients were established as previously described.[7, 8] Briefly, the surface of the ovary was scraped gently, and the epithelial cells were then plated in a 1:1 mixture of MCDB 105/M199 medium supplemented with 15% heat-inactivated fetal bovine serum and epidermal growth factor (10 ng/ml). Cells were cultured at 37° C in 5% carbon dioxide and 95% humidified air.

Collection of conditioned culture medium. Each of the ovarian cancer cell lines and normal ovarian epithelial cultures were grown to confluence in their respective culture media containing 10% fetal bovine serum. After washing the cells with Hank's buffered salt solution, the cells were grown for 48 hours in serum-free medium. The cancer cell lines were grown in Richter's serum-free medium,[9] and the normal epithelial cells were grown in serum-free MCDB 105/M199 medium without epidermal growth factor. After 48 hours, the conditioned medium was removed and stored at −70° C. Before testing conditioned media for the presence of TGF-β, media from each of the cells was divided into two aliquots; one of which was heated at 90° C for 10 minutes while the other was untreated. The conditioned media were then dialyzed, lyophilized, and reconstituted at a fivefold increased concentration in medium containing 2% fetal bovine serum. The reconstituted media each were added to six replicate wells of CCL-64 cells, which are known to be growth inhibited by TGF-β,[10] to test for the presence of active TGF-β. Serum-free media that had not been exposed to cells was processed in an identical manner and used as a negative control. Authentic porcine TGF-β_1 (R & D Systems, Minneapolis, Minn.) was used as a positive control.

Immunohistochemistry. Frozen sections were obtained from three normal human ovaries that were removed at the time of hysterectomy for benign gynecologic diseases. Immunohistochemical staining for TGF-β was performed with the Vectastain ABC kit (Vector Laboratories, Burlingame, Calif.) as described previously.[11] Monoclonal antibody ID11.16 (Collagen Corporation, Palo Alto, Calif.) which recognizes the mature forms of both TGF-β_1 and TGF-β_2, was used as the primary antibody (5 μg/ml).[12] Purified mouse immunoglobin G specific for nonhuman tissue (Coulter Immunology, Hialeah, Fla.) was used as a negative control (1:100 dilution). Anticytokeratin AE1/AE3 (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was used as a positive control. The slides were developed for 4 minutes with the enzyme substrate diaminobenzidine (0.5% diaminobenzidine in 0.05% Tris buffer, 0.6% hydrogen peroxide). Slides then were rinsed in water, counterstained with methyl green, dehydrated, and mounted.

[^{3}H]thymidine assay. Each of the five ovarian cancer cell lines and CCL-64 cells were grown in supplemented modified Eagle's medium containing 2% fetal bovine serum in 96-well culture plates (5×10^4 cells/well). Normal ovarian epithelial cells were grown in MCDB 105/M199 medium containing 2% FBS. Authentic porcine TGF-β_1 (0, 0.2, 2, 10, or 20 ng/ml) (R & D Systems, Minneapolis, Minn.) or antibody was added at the time of plating to six replicate wells containing CCL-64 cells. Anti-TGF-β antibodies used included a polyclonal antisera (R & D Systems) and monoclonal antibody 1D11.6 (Collagen Corporation). Both of these reagents neutralize TGF-β_1 and TGF-β_2. In several experiments, A2C6 (Centocor, Malvern, Pa.), a murine monoclonal antibody raised against a human hepatitis B virus, was used as a negative control. The cells were grown for 48 hours. During the last 4 hours of culture, 1 μCi of [^{3}H]thymidine (6.7 Ci/mmol/L) was added to each well. The culture medium was then removed. Cells were washed three times with phosphate-buffered saline solution and harvested. Incorporated radioactivity was measured in a Packard liquid scintillation counter.

Ribonuclease protection assay. Cells were grown to confluence in medium containing 10% fetal bovine serum. Ribonucleic acid (RNA) was prepared after lysis of cells in a hypotonic solution with the nonionic detergent Nonidet P-40 supplemented with 200 μg/ml heparin (Sigma Chemical Co., St. Louis, Mo.) as a ribonuclease inhibitor. RNA was quantitated by absorbance at 260 nm. Analysis of total RNA by the ribonuclease protection assay was performed according to published protocol, with some optimizing changes.[13] Briefly, 10 μg cellular RNA plus 20 μg transfer RNA

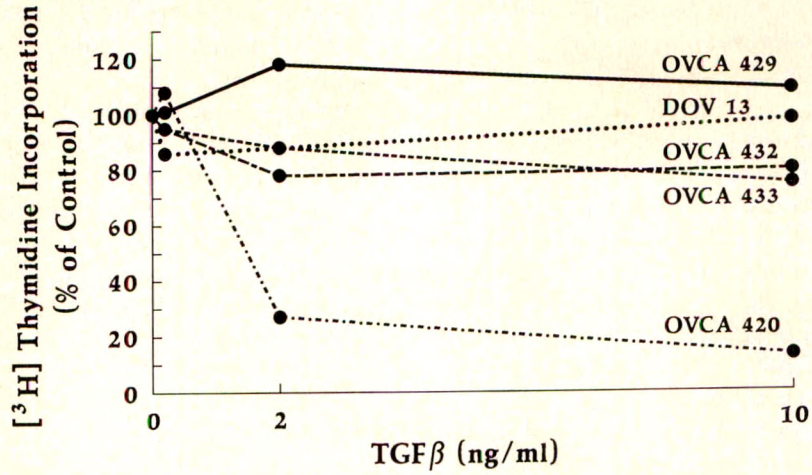


Fig. 1. Effect of TGF-β on proliferation of ovarian cancer cell lines (OVCA 420, $p < 0.01$; OVCA 432, OVCA 433, $p < 0.05$; OVCA 429, DOV 13, not significant).

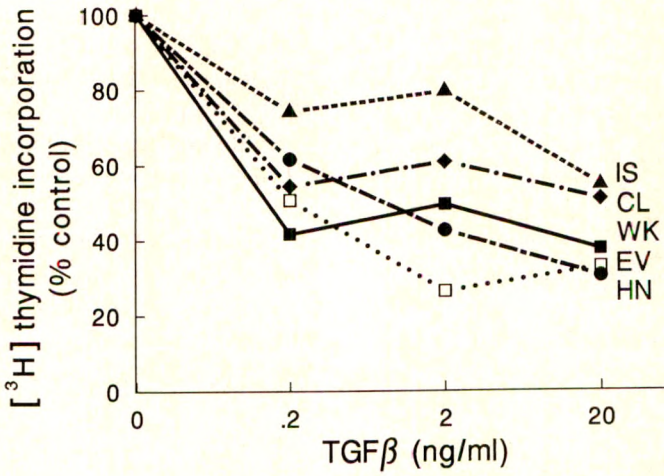


Fig. 2. Effect of TGF-β on proliferation of normal human ovarian epithelial cells ($p < 0.01$).

was combined with phosphorus 32–labeled complementary RNA (1.5 to 2 × 10^5 counts/min) in 30 μl hybridization buffer, heated to 85° C for 5 minutes, and then incubated at 53° C overnight. Ribonuclease digestion was carried out by the addition of 350 μl of ice-cold digest buffer, containing 0.24 units/ml ribonuclease A and 80 units/ml ribonuclease T_1, and proceeded for 30 minutes at 35° C. Ribonuclease A and T_1 and transfer RNA were from Boehringer Mannheim (Indianapolis, Ind.). Samples were then treated with proteinase K, extracted with phenol/chloroform, and precipitated with ethanol. Samples were dissolved in gel-loading buffer and fractionated by electrophoresis with 4.5% polyacrylamide-6 mol/L urea sequencing gels, each lane containing the equivalent of 6 μg of inital cellular RNA. Control samples, processed concurrently with authentic samples, contained transfer RNA alone (negative control) or transfer RNA plus a mixture of sense complementary RNAs prepared by in vitro transcription of complementary DNA for TGF-β_1, β_2, and β_3 positioned downstream of the SP6 promoter (positive control). The use of sense complementary RNAs containing full-length coding sequences for each of the three human TGF-βs demonstrated the lack of cross hybridization between the three TGF-β probes (data not shown). The preparation of TGF-β probes for the ribonuclease protection assay has been described previously.[14] RNA probes were stored in water at −70° C and used within 4 to 7 days of preparation. For ribonuclease protection assays, probes for TGF-β_1 and β_2 were mixed together (1.5 × 10^5 counts/min each/sample) since the diagnostic ^{32}P-labeled fragments

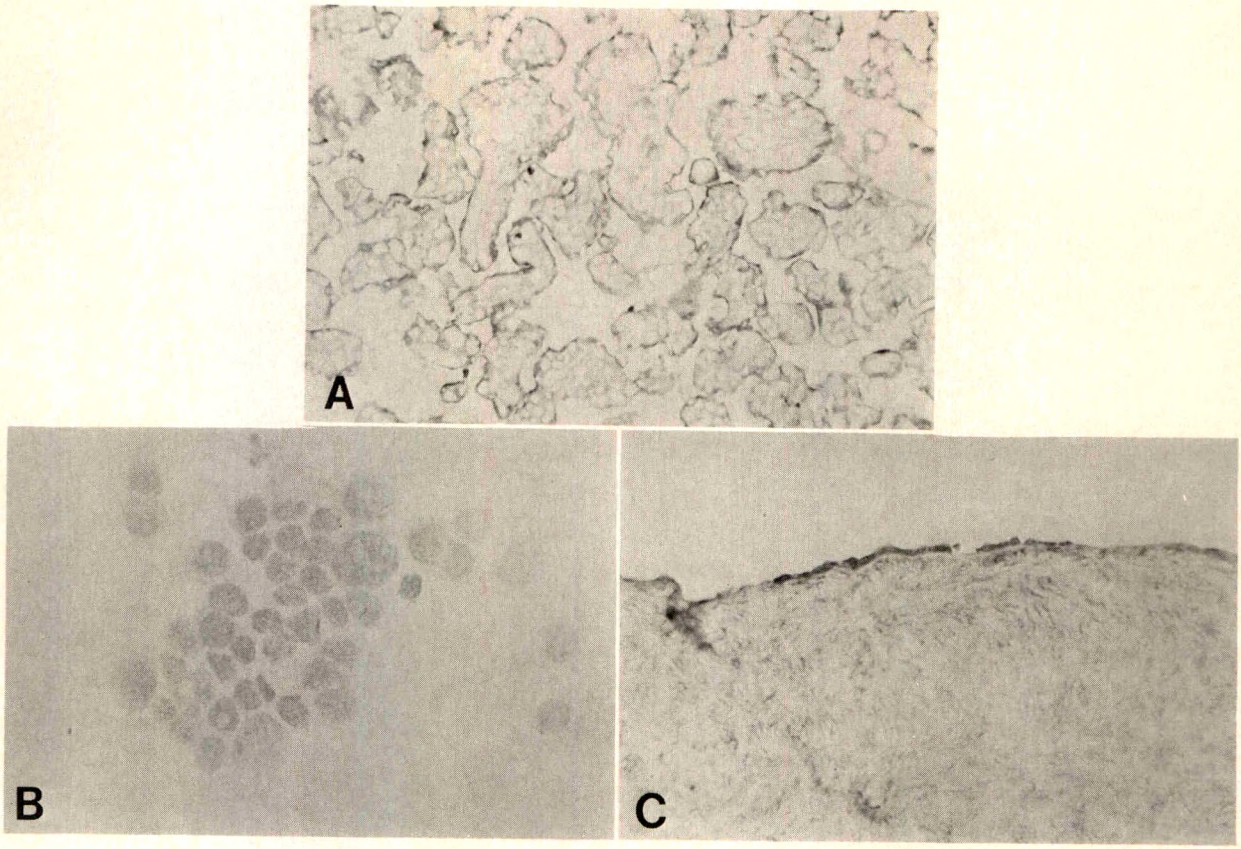


Fig. 3. Immunohistochemical staining for TGF-β. **A,** Placenta (positive control). **B,** OVCA 420 cells. **C,** Normal human ovary.

could be easily distinguished by size. The probe for TGF-β_3 was hybridized with samples separately (2×10^5 counts/min/sample).

Statistics. The Student *t* test was used to analyze experiments in which mean values were compared.

Results

The effect of TGF-β on [^{3}H]thymidine incorporation of the five ovarian cancer cell lines and normal ovarian epithelial cells is shown in Figs. 1 and 2. TGF-β markedly inhibited [^{3}H]thymidine incorporation (>90%) in OVCA 420 cells ($p < 0.01$), whereas OVCA 432 and OVCA 433 cells were only modestly inhibited (15% to 20%) ($p < 0.05$). Proliferation of OVCA 429 and DOV 13 cells was unaffected by TGF-β (Fig. 1). In normal ovarian epithelial cells from five patients, TGF-β inhibited incorporation of [^{3}H]thymidine by >40% in all cases ($p < 0.01$) (Fig. 2). In all experiments, TGF-β (2 to 10 ng/ml) inhibited [^{3}H]thymidine incorporation of CCL64 cells (the positive control) by >95% (data not shown).

Immunohistochemical staining for TGF-β with a monoclonal antibody reactive with both TGF-β_1 and TGF-β_2 is shown in Fig. 3. In Fig. 3, *A*, staining for TGF-β is seen in placenta, which is known to produce large amounts of TGF-β (positive control). In Fig. 3, *B*, immunohistochemically detectable TGF-β is seen in a cytospin preparation of OVCA 420 cells. Staining for TGF-β also was seen in each of the other ovarian cancer cell lines except OVCA 432 (data not shown). In Fig. 3, *C*, immunoreactive TGF-β is seen in the epithelium in a frozen section of normal human ovary. Immunohistochemically detectable TGF-β also was seen in cytospin preparations of cultured normal ovarian epithelial cells.

Expression of TGF-β messenger RNA by the ovarian cancer cell lines is shown in Figs. 4 and 5. TGF-β_1 and TGF-β_2 messenger RNA were present in OVCA 420, OVCA 429, OVCA 433, and DOV 13 cells (Fig. 4). In contrast, OVCA 432 cells do not contain TGF-β_1 messenger RNA and only a faint TGF-β_2 band is seen. In Fig. 5, TGF-β_1 and TGF-β_2 transcripts are seen in RNA derived from normal ovarian epithelial cells from two patients. In addition, the appropriate positive and negative controls that were described in the material and methods section are shown in Fig. 5. Although a TGF-

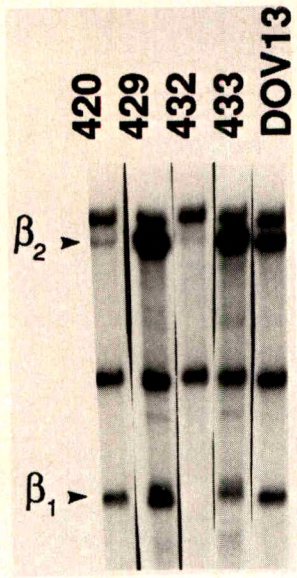


Fig. 4. TGF-β messenger RNA expression in ovarian cancer cell lines.

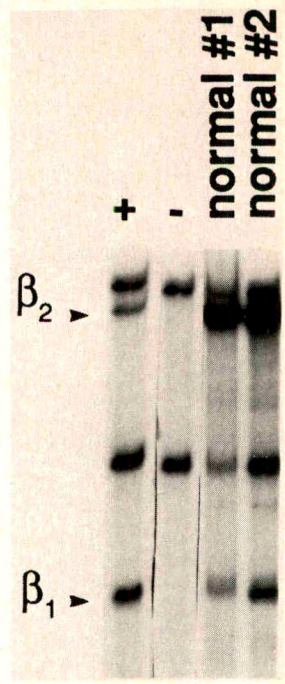


Fig. 5. TGF-β messenger RNA expression in normal ovarian epithelial cells from two patients along with positive and negative controls.

β_3 band was seen in the lane representing the positive control, none of the ovarian cancer cell lines or normal ovarian epithelial cells were found to produce TGF-β_3 messenger RNA (data not shown).

Previously, we have shown that among the five ovarian cancer cell lines, only OVCA 433 cells secrete biologically active TGF-β.[15] In the current study, we examined whether the ovarian cancer cell lines secrete latent TGF-β that can be activated by heating. The effect of fivefold concentrated heated conditioned culture medium from each of the ovarian cancer cell lines except DOV 13 (not done) on [^{3}H]thymidine incorporation of CCL64 cells is compared with that of unheated medium (Fig. 6). Heating resulted in generation of TGF-β–like activity in conditioned media from all but one of the ovarian cancer cell lines relative to unheated media, which is respresented as 100% of control in Fig. 6 ($p < 0.05$). OVCA 432, which does not produce appreciable amounts of TGF-β messenger RNA transcripts or immunohistochemically detectable TGF-β, also was not found to produce latent TGF-β with this bioassay.

We also tested heated and unheated serum-free conditioned medium from normal ovarian epithelial cultures from two patients for the presence of active and latent TGF-β. We found that fivefold concentrates of unheated medium conditioned by epithelial cells from two normal ovaries consistently decreased [^{3}H]thymidine incorporation of CCL64 cells ($p < 0.05$). When the medium was heated before exposure to CCL64 cells, to activate latent TGF-β, an even greater inhibition of proliferation was noted (Fig. 7). In addition, the inhibition caused by the conditioned medium was reversible if anti-TGF-β monoclonal antibody (10 μgm/ml) was added to the cultures. Serum-free medium that had not been exposed to cells but which had been processed in an identical manner had no effect on [^{3}H]thymidine incorporation of CCL-64 cells (data not shown).

In Fig. 8, the effect of polyclonal anti-TGF-β antisera on [^{3}H]thymidine incorporation of four of the ovarian cancer cell lines is shown. Proliferation of three ovarian cancer cell lines that either do not express TGF-β (OVCA 432) or express only latent TGF-β (OVCA 420, OVCA 429) was not affected by the antibody. On the other hand, proliferation of OVCA 433 cells, which both secrete active TGF-β and are inhibited by exogenous TGF-β, was stimulated by the anti-TGF-β antisera ($p < 0.05$). Similar results were obtained using the monoclonal anti-TGF-β antibody (data not shown). In addition, the monoclonal anti-TGF-β antibody significantly stimulated ($p < 0.01$) [^{3}H]thymidine incorporation of normal ovarian epithelial cells from one patient (Fig. 9), whereas ovarian epithelial cells derived from another patient were not stimulated. These experiments were repeated several times with different concentrations of serum (0% to 10%), different cell densities (confluent, subconfluent), and different incubation times (24 to 48 hours). Optimal stimulation of proliferation was noted in confluent cells after 48 hours in serum-free medium. The negative control antibody A2C6 had no effect on [^{3}H]thymidine incorporation of

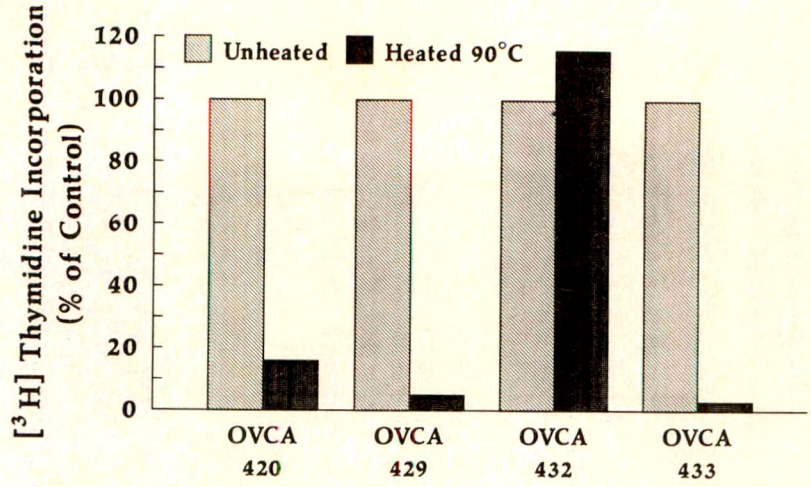


Fig. 6. Production of latent TGF-β by epithelial ovarian cancer cell lines (OVCA 420, OVCA 429, OVCA 433, $p < 0.05$; OVCA 432, not significant).

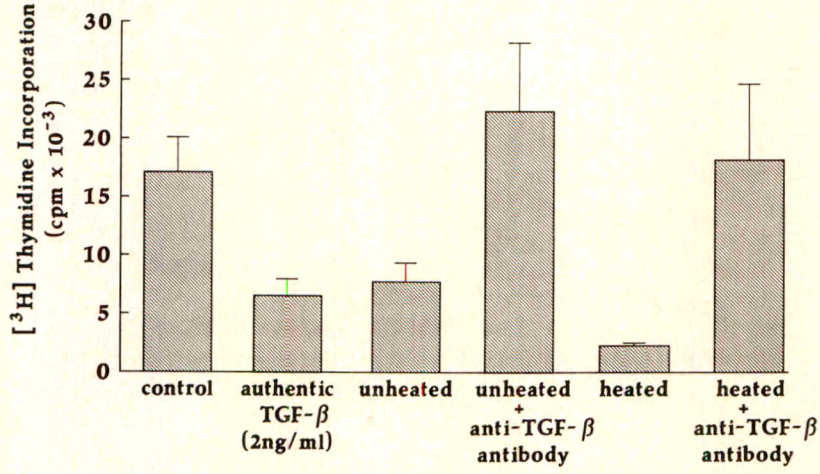


Fig. 7. Production of latent and active TGF-β by human ovarian epithelial cells (heated, unheated, $p < 0.05$).

the cancer cell lines or the normal epithelial cells (data not shown).

Comment

Although TGF-β was discovered due to its ability to act in concert with TGF-α to transform some types of normal cells in culture,[1] TGF-β has been shown to inhibit proliferation of many normal epithelial cells.[2] In addition, TGF-β also frequently induces differentiation of epithelial cells in culture. For example, bronchial epithelial cells undergo terminal differentiation to a squamous phenotype after exposure to TGF-β.[16] The mechanism of action of TGF-β is poorly understood, however. TGF-β receptors have not been well characterized and the intracellular events that are triggered after TGF-β binding have not been defined. Although in vitro evidence suggests that TGF-β might be a physiologically important growth inhibitory substance, the role of TGF-β in normal processes of growth and differentiation remains unclear.

The potential role of TGF-β as a growth inhibitory substance has been studied extensively in breast epithelium. It has been shown that normal breast epithelial cells produce TGF-β and that TGF-β acts to inhibit proliferation of these cells.[17, 18] In addition, it has been shown that steroid hormones that inhibit growth of normal breast epithelium, such as progestins and antiestrogens, stimulate production of TGF-β.[19] Conversely, estrogens, which stimulate growth, act to inhibit TGF-β production.[14, 19] It has been proposed that the effect of circulating steroid hormones on proliferation of breast epithelium may be due to induction or suppression of production of TGF-β and other peptide growth factors.

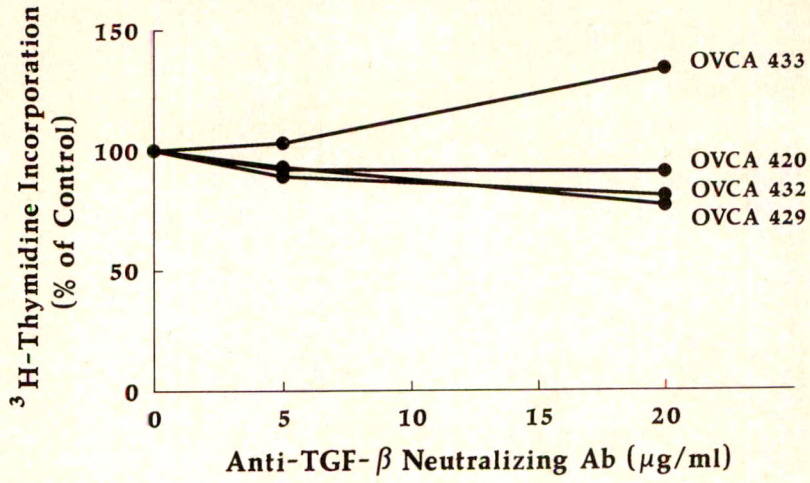


Fig. 8. Effect of polyclonal anti-TGF-β antisera on proliferation of epithelial ovarian cancer cell lines (OVCA 433, $p < 0.05$).

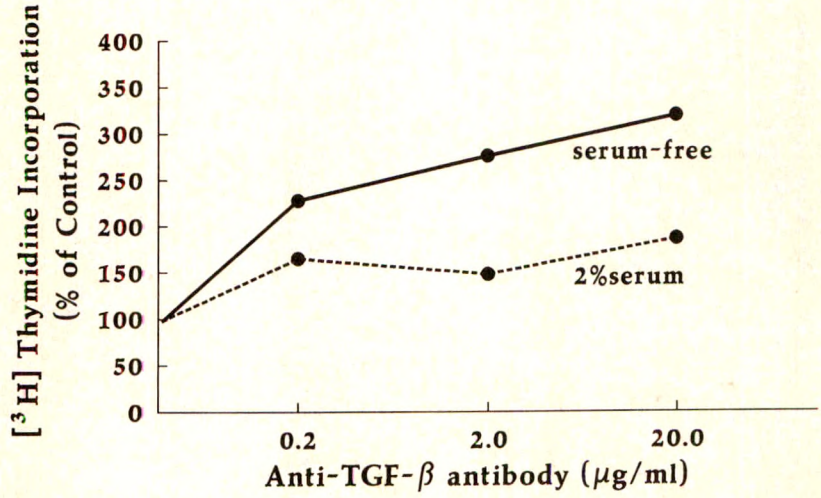


Fig. 9. Effect of monoclonal anti-TGF-β antibody on proliferation of normal ovarian epithelial cells (serum-free, 2% serum, $p < 0.05$).

In the present study we found that, like normal breast epithelium, normal ovarian epithelium produces both TGF-β_1 and TGF-β_2 and is growth-inhibited by exogenous TGF-β. These data suggest that TGF-β might act in vivo as an autocrine growth inhibitory substance that prevents inappropriate proliferation of ovarian epithelial cells, which normally are thought to proliferate relatively slowly. At the time of ovulation, this inhibitory pathway might be turned off to allow proliferation of epithelial cells to cover the defect that is caused by extrusion of the ovum through the ovarian surface. Because the levels of estrogens and progesterone in the ovary vary markedly during the cycle, we are investigating the possibility that TGF-β production by ovarian epithelial cells is regulated by steroid hormones in a manner similar to that observed in breast epithelium. In addition, our group[8] and Siemens and Auersperg[20] have shown that epidermal growth factor stimulates proliferation of normal human ovarian epithelial cells. Proliferation of ovarian epithelial cells after ovulation, or at other times, might be due to both a decrease in growth inhibitory pathways such as TGF-β and increased availability of mitogenic peptide growth factors.

We found that normal human ovarian epithelial cells sometimes, but not always, appeared to activate the latent TGF-β that they secreted into their culture medium. Further studies are needed to determine whether TGF-β produced by ovarian epithelial cells is activated in vivo. In addition, the mechanism of activation and the cell types involved also must be identified. In this regard, it is known that TGF-β is secreted from cells in a biologically inactive form noncovalently

bound to a portion of the precursor molecule from which it is cleaved during processing.[2] Furthermore, TGF-β in serum is bound to α_2-macroglobulin.[21] Although TGF-β can be activated by low pH or heating in vitro,[22] these are not thought to be physiologically relevant mechanisms of TGF-β activation. There is some evidence to suggest that proteases and glycosidases may play an important growth regulatory role in vivo by liberating active TGF-β from its precursor.[23, 24] If a cell that produces TGF-β and responds to TGF-β also produced the enzymes that activate TGF-β, it would possess all of the components of an autocrine growth inhibitory loop. On the other hand, if a cell lacked the enzymes needed to activate TGF-β, it is possible that TGF-β still might be activated in the local environment if these enzymes were produced by other nearby cells. This latter hypothesis is particularly appealing because the ovary and other human organs are comprised of several different cell types that presumably must communicate to act in a coordinated fashion.

Although increased proliferation associated with cancer may be due to inappropriate production of growth stimulatory factors, loss of growth inhibitory pathways, such as TGF-β, also could result in increased proliferation. In this regard, we found that all but one of the ovarian cancer cell lines were either partially or completely resistant to the growth inhibitory effect of TGF-β relative to normal ovarian epithelial cells. Similarly, several other transformed cells and human cancer cells that are resistant to TGF-β have been described.[25-27] In addition, although normal ovarian epithelial cells and most of the ovarian cancer cell lines produced TGF-β_1 and TGF-β_2, one of the ovarian cancer cell lines produced little, if any, TGF-β. Finally, only one of the ovarian cancer cell lines was able to activate the TGF-β that it produced. These findings suggest that loss of various components of the TGF-β pathway is common in ovarian cancer cell lines. It is possible that loss of this growth inhibitory pathway might play a role in the development of some ovarian cancers.

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Prostaglandin- or endothelium-mediated vasodilation is not involved in the blunted responses of blood vessels to vasoconstrictors in pregnant rats

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Pregnancy is associated with decreases of blood pressure and vascular sensitivity to vasopressor agents. We have hypothesized that the increased liberation of endogenous vasodilator(s) by the vascular endothelium or other structures could mediate these blunted responses. Thoracic aorta rings of nonpregnant, 21 days pregnant, and first day post partum rats respond similarly to acetylcholine, an endothelium-dependent vasorelaxant. In contrast, the potency of the response to sodium nitroprusside, an endothelium-independent vasorelaxant, is unchanged in tissues of pregnant rats and increased ($p < 0.05$) in those of post partum animals. In the presence of indomethacin (10 μmol/L) the three groups of tissues show a decreased potency. The effects of phenylephrine on aortic rings of both nonpregnant and pregnant rats are markedly increased in the presence of N^{g}-monomethyl-L-arginine. Indeed, the concentration producing 50% of the maximum response of phenylephrine decreases ($p < 0.001$) from 50.7 to 8.02, from 93.8 to 37.6, and from 60.4 to 5.97 nmol/L with the use of N^{g}-monomethyl-L-arginine (0.1 mmol/L) in rings from nonpregnant, pregnant, and postpartum rats, respectively. Simultaneously, the maximum response to phenylephrine increases markedly in the three groups of tissues. In the presence of N^{g}-monomethyl-L-arginine, indomethacin does not influence the response to phenylephrine. Our results do not support the possible involvement of an endogenous vasodilator (prostaglandin-like or endothelium-derived) in the blunted responses to vasoconstrictors during pregnancy. (Am J Obstet Gynecol 1992;166:684-92.)

Key words: Pregnancy, vasoconstriction, endothelium-derived relaxation, prostaglandins

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6/1/34366

In recent years, it has become clear that the endothelial lining of blood vessels performs many physiologic functions. Such an influence was first demonstrated by Furchgott and Zawadsky,[1] accounting for the vascular smooth muscle–relaxing effect of acetylcholine. It has also become evident that endothelium-dependent relaxation modulates the effects of vasoconstrictor agents[2] and mediates flow-dependent relaxation.[3] Some inhibitors of this endothelium-dependent relaxation have become available recently as, for example, N^{g}-monomethyl-L-arginine and L-argininosuccinic acid.[4]

Under physiologic conditions, such as pregnancy,

blood pressure decreases,[5, 6] possibly as a consequence of the decreased sensitivity of blood vessels to vasoconstrictor agents. This reduced sensitivity has been noted in many tissues and in some vascular beds of pregnant rats such as aorta,[7] portal vein,[8] caudal artery,[9] and pulmonary[10] and mesenteric[8] beds. This effect is not specific for a given agent, because it was observed with several vasoconstrictors, such as angiotensin, vasopressin, norepinephrine, etc.[8] This decreased sensitivity of blood vessels of pregnant rats is similar in magnitude to the blunted pressor responses to vasoconstrictors observed in conscious pregnant rats.[11-13]

The vasoconstrictor effect of many agents is reported to be diminished in the presence of the endothelium in isolated vessels.[14] Because we have recently reported that the sensitivity of mesenteric artery rings of both nonpregnant and pregnant rats to α-adrenergic agents is dependent on the functional presence of the endothelium,[15] we undertook the present experiments to further investigate the involvement of the endogenous vasorelaxant influence of the vessel wall in the blunted responses to vasoconstrictors at the end of pregnancy and in the postpartum period in the rat. In these experiments, we look particularly at endothelium-derived relaxing factor (EDRF) and arachidonate derivatives through the cyclooxygenase pathway. The end of pregnancy (twenty-first day) and postpartum period were chosen because, under these conditions, important changes occur in vascular reactivity.[7]

Methods

Ten-week-old female Sprague-Dawley rats, obtained from Charles River Canada (St-Constant, Québec), were mated with males of the same age. The morning on which vaginal smears were found to contain spermatozoa was labeled day 1 of gestation. The experiments were performed on the twenty-first day of pregnancy and between 18 and 36 hours after parturition (post partum). Unmated, age-matched (13 to 14-week-old) females served as controls. After the rats were decapitated, the thoracic aorta was rapidly removed and placed in cold Krebs solution. The aortic tube was freed from surrounding tissues and cut transversally to obtain rings 3 to 4 mm in axial length. Small hooks, made with 30-gauge needles, were carefully inserted into each ring to suspend them vertically in 15-ml glass-jacketed organ baths. The tissues were attached to isometric force-displacement transducers (FT-03, Grass Instruments, Quincy, Mass.) connected to a #7 Grass Polygraph. They were stretched to a passive tension of 2.0 gm, which, in preliminary experiments (Parent and St-Louis, unpublished data), was found to be optimal for aortic rings of both nonpregnant and pregnant rats. The Krebs solution used in these experiments contained in millimoles per liter: sodium chloride, 118; sodium bicarbonate, 25; potassium chloride, 4.7; calcium chloride, 2.5; magnesium sulfate, 1.18; potassium phosphate, 1.18; and dextrose, 5.5. The pH was 7.4 at 37° C, and the solution was bubbled with a mixture of 95% oxygen:5% carbon dioxide.

After 1 hour of equilibration, phenylephrine (1 μmol/L) was added to the bathing solution to measure tissue reactivity. At the plateau response of phenylephrine, the functional integrity of the endothelium was assessed by the application of acetylcholine (0.1 mmol/L). This response was quantified as the percent relaxation of phenylephrine-induced contraction (i.e., relaxation from the plateau response to phenylephrine). After full relaxation (45 to 50 minutes), the different experimental protocols were done. In the first protocol, we measured the response to acetylcholine, an endothelium-dependent vasorelaxant, in aortic rings of nonpregnant, pregnant, and post partum rats. A cumulative concentration-response curve to phenylephrine (1 nmol/L to 10 μmol/L) was charted for the tissues of each group of rats. The rings were then washed to remove phenylephrine by frequent changes in bathing fluid, and 75 to 90 minutes later a cumulative concentration-response curve to acetylcholine (10 nmol/L to 100 μmol/L) was obtained after precontracting rings with phenylephrine. The phenylephrine concentration used to precontract the tissues was 1.0 μmol/L, which produces an increase in tissue tension of about 80% to 90% of maximum response in rings of the different groups. A similar protocol was used for a second set of tissues, except that the curve to acetylcholine was replaced by a cumulative concentration-response curve to sodium nitroprusside (1 nmol/L to 10 μmol/L).

In a second protocol, a cumulative concentration-response curve to phenylephrine was first measured in the absence and then, on the same tissue, in the presence of N^G-monomethyl-L-arginine (0.10 mmol/L) or indomethacin (10 μmol/L) to block the influence of EDRF and of endogenous derivatives of arachidonic acid, respectively. Similar groups of rats were used (nonpregnant, pregnant, and post partum).

Each experimental concentration-response curve was analyzed as previously[7, 8] by microcomputer using the ALLFIT program to determine the concentration producing 50% of the maximum response and the maximum asymptote of the response. Statistical comparisons were made on the mean negative logarithm of the concentration producing 50% of the maximum response and mean maximum asymptote for each experimental group. Analysis of variance was performed, followed by the Dunnett test to compare each pregnant and post partum group with the nonpregnant controls. The paired *t* test was also performed to compare the concentration-response curve in the absence and in the presence of inhibitors in each experimental group. Analysis of variance with a two-factor mixed design and

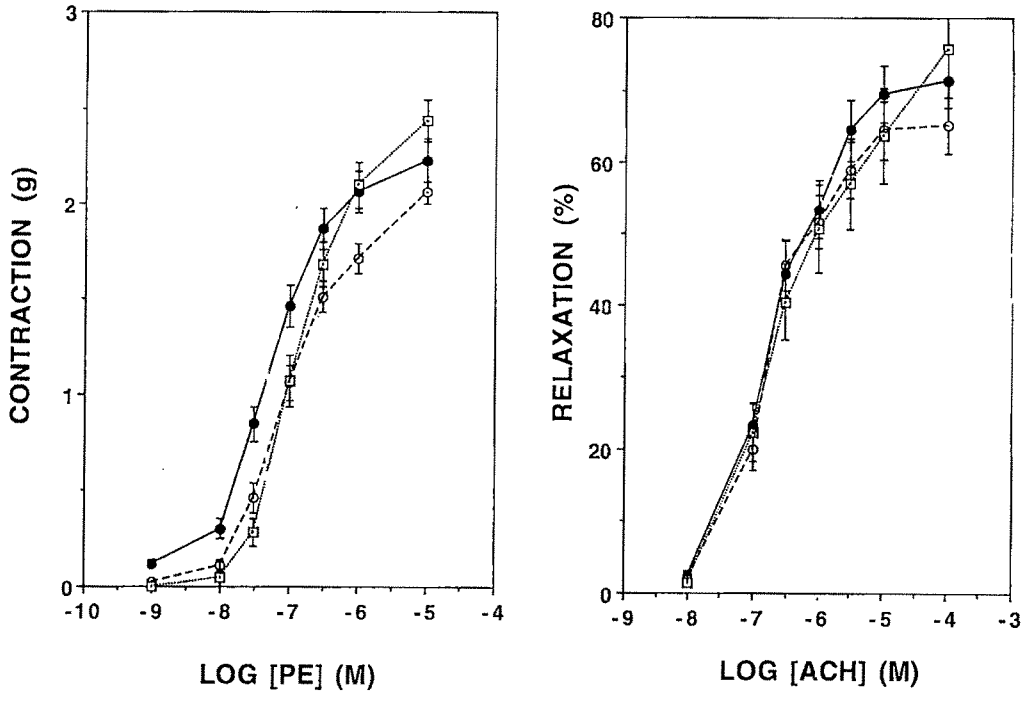


Fig. 1. Concentration-response curves to phenylephrine *(left)* and acetylcholine *(right)* in aortic rings of nonpregnant *(filled circles)*, pregnant *(open circles)*, and post partum *(open squares)* rats. Symbols and bars represent the means ± SE of number of measurements shown in Table I. Abscissa indicates molar concentration of agonists in logarithm and ordinate, contractile responses in grams for phenylephrine *(left panel)*, and the percent relaxation from precontracted level (phenylephrine, 1 μmol/L) for acetylcholine *(right panel)*.

Table I. Sensitivity and responsiveness to phenylephrine and acetylcholine in aortic rings of nonpregnant, pregnant and postpartum rats

		Phenylephrine			*Acetylcholine*		
Group	*n*	*Negative log of concentration producing 50% of maximal response*	*50% of maximal response (nmol/L)*	*Maximum response (gm)*	*Negative log of concentration producing 50% of maximal response*	*50% of maximal response (μmol/L)*	*Maximum response (%)*
Nonpregnant	20	7.26 ± 0.06	54.9	2.22 ± 0.10	6.60 ± 0.09	0.25	71 ± 4
Pregnant	36	6.96 ± 0.08*	110	2.04 ± 0.05*	6.68 ± 0.08	0.21	64 ± 4
Postpartum	16	6.85 ± 0.07*	141	2.38 ± 0.10*	6.57 ± 0.11	0.27	78 ± 3

n, number of tissues tested.
*$p < 0.01$, evaluated by the Dunnett test.

repeated measure of one factor[13] were also used for the indomethacin and N^g-monomethyl-L-arginine results.

The agents used were L-phenylephrine hydrochloride and acetylcholine chloride obtained from Sigma Chemical Co. (St. Louis, Mo.), sodium nitroprusside from Fisher Scientific (Montreal Quebec), N^g-monomethyl-L-arginine monoacetate from Calbiochem Biochemicals (San Diego, Calif.), and indomethacin lactose from Merck Frosst (Pointe-Claire, Quebec).

Results

The effects of phenylephrine and acetylcholine on aortic rings from nonpregnant, pregnant, and post partum rats are reported in Fig. 1 and Table I. The responses to phenylephrine were markedly decreased in pregnant and post partum tissues, as shown by the rightward shift of their concentration-response curves in comparison with the controls (Fig. 1); the maximum responses were only slightly modified. These results, reported in Table I, show that the concentration elic-

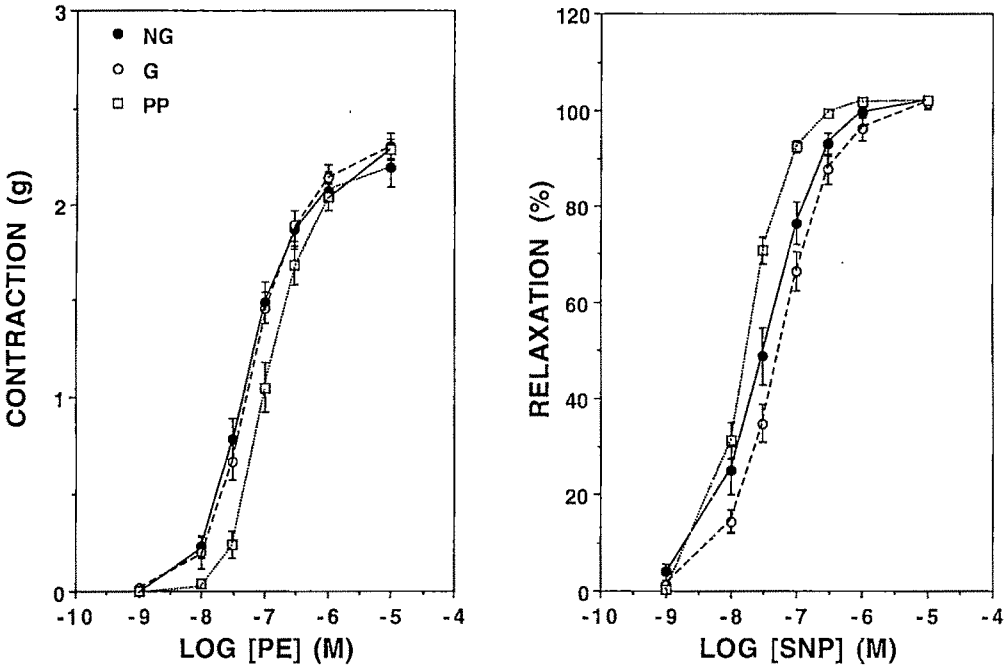


Fig. 2. Concentration-response curves to phenylephrine *(left)* and sodium nitroprusside *(right)* in aortic rings of nonpregnant *(filled circles)*, pregnant *(open circles)*, and post partum *(open squares)* rats. Symbols and bars represent means ± SE of number of measurements shown in Table II. Abscissa indicates molar concentration of agonists in logarithm and ordinate, contractile responses in grams for phenylephrine *(left panel)*, and percent relaxation from precontracted level (phenylephrine, 1 μmol/L) for sodium nitroprusside *(right panel)*.

Table II. Sensitivity and responsiveness to phenylephrine and sodium nitroprusside in aortic rings of nonpregnant, pregnant, and postpartum rats

		Phenylephrine			*Sodium nitroprusside*		
Group	*n*	*Negative log of concentration producing 50% of maximal response*	*50% of maximal response (nmol/L)*	*Maximum response (gm)*	*Negative log of concentration producing 50% of maximal response*	*50% of maximal response (nmol/L)*	*Maximum response (%)*
Nonpregnant	23	7.29 ± 0.06	51.7	2.13 ± 0.09	7.52 ± 0.10	30.0	101.7 ± 1.1
Pregnant	20	7.14 ± 0.04*	72.7	2.25 ± 0.08†	7.38 ± 0.06	42.2	101.9 ± 0.8
Postpartum	20	6.56 ± 0.05*	183	1.83 ± 0.07*	7.79 ± 0.09†	16.4	104.9 ± 1.1

n, number of tissues tested.
*$p < 0.01$, evaluated by the Dunnett test.
†$p < 0.05$, evaluated by the Dunnett test.

iting 50% maximum response of phenylephrine was significantly ($p < 0.01$) increased by a factor of 2 and 2.6 in (ring of) pregnant and postpartum rats, respectively, compared with nonpregnant rats, whereas the maximum response was significantly ($p < 0.01$) decreased in pregnant and elevated in postpartum animals. The vasorelaxant responses to acetylcholine were similar in both groups of experimental tissues relative to nonpregnant rats, in terms of concentration producing 50% maximum response and maximum response.

Fig. 2 shows the results of an identical protocol with sodium nitroprusside, an endothelium-independent vasorelaxant. The responses to phenylephrine were again decreased in pregnant and post partum tissues in comparison with nonpregnant controls (Table II). The sensitivity of the vasorelaxant response to sodium nitroprusside was increased in tissues of post partum rats compared with their nonpregnant counterparts but not modified in the pregnant rats (Fig. 2, Table II). In contrast to acetylcholine, sodium nitroprusside fully re-

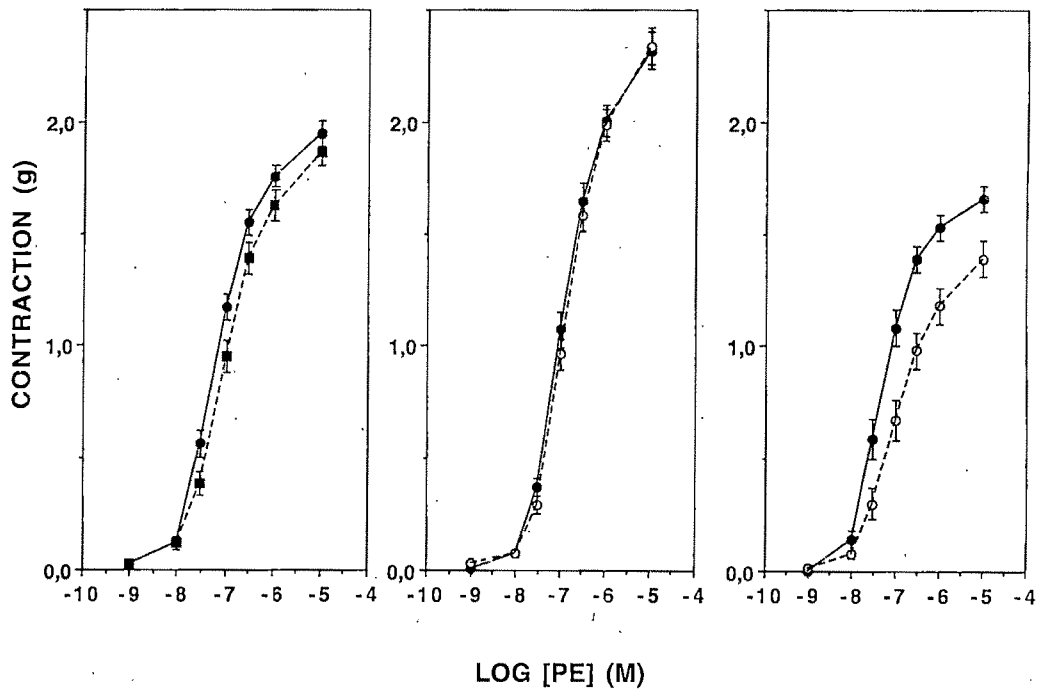


Fig. 3. Concentration-response curves to phenylephrine in absence *(filled circles)* and presence *(open circles)* of indomethacin (10 μmol/L) in aortic rings of nonpregnant *(left)*, pregnant, *(middle)*, and post partum *(right)* rats. Symbols and bars represent means ± SE of number of measurements shown in Table III. Abscissa indicates molar concentration of phenylephrine in logarithm and ordinate, contractile responses in grams.

laxed the tissues from the precontracted level induced by 1.0 μmol/L phenylephrine. The maximum responses to sodium nitroprusside were identical in all groups of tissues and were not statistically different from 100% relaxation.

Fig. 3 shows the effect of 10 mol/L indomethacin on concentration-response curves to phenylephrine. We have previously observed, by mass spectroscopy coupled to gas chromatography, that this concentration of indomethacin (10 μmol/L) totally inhibited the output of prostaglandin material in experimental conditions similar to the ones used here (Falardeau and St-Louis, unpublished data). In the present experiments, indomethacin decreased the responsiveness of nonpregnant and post partum aortic rings to phenylephrine (Fig. 3, *left* and *right panels*, respectively), as illustrated by the rightward shift of the concentration-response curves and the depression of the maximum response in post partum rats. On the other hand, it did not substantially affect the response to phenylephrine in tissues from pregnant rats (Fig. 3, *middle panel*). Significant decreases in the negative logarithm of the concentration required for 50% maximum response were observed in tissues of all groups (Table III), but the magnitude of this effect was much less in pregnant rats. The maximum responses were decreased in nonpregnant and postpartum tissues ($p < 0.001$), suggesting that an arachidonic acid derivative was secreted on stimulation with phenylephrine and that it exerted contractile properties on the aortic rings.

In a similar protocol we tested the blockade of EDRF liberation by N^g-monomethyl-L-arginine. Fig. 4 shows that N^g-monomethyl-L-arginine (0.1 mmol/L) substantially increased the responses of the aortic rings in the three groups of animals. This concentration of N^g-monomethyl-L-arginine (0.1 mmol/L) was shown to almost totally abolish the vasorelaxant response to carbachol in phenylephrine-precontracted aortic rings of the rat (St-Louis and Sicotte, preliminary experiments). This was manifested by both an augmented potency (leftward shift of the concentration-response curves) and increased maximum responses. These effects were highly significant (Table IV) for the three groups of tissues, but the increase in potency was of a smaller magnitude in pregnant animals than in nonpregnant and post partum rats, whereas the enhancement of the maximum response was similar in all three tissue types. This is demonstrated by smaller increases in the negative logarithm of the concentration producing 50% maximum response values in pregnant than in nonpregnant and post partum tissues, which are respectively 0.39 ± 0.04, 0.80 ± 0.09, and 1.05 ± 0.10 units of the negative logarithm of the concentration producing 50% maximum response (all $p < 0.001$). These observations suggest that, in intact aortic rings of the rat, the effect of phenylephrine is

Table III. Effect of indomethacin on sensitivity and responsiveness to phenylephrine in aortic rings of nonpregnant, pregnant, and postpartum rats

		Control		*Indomethacin*	
Group	*n*	*Negative log of concentration producing 50% of maximal response (nmol/L)**	*Maximal response (gm)*	*Negative log of concentration producing 50% of maximal response (nmol/L)**	*Maximal response (%)*
Nonpregnant	20	7.36 ± 0.06 (43.7)	1.77 ± 0.09	7.06 ± 0.08 (86.8) $p < 0.001$†	1.59 ± 0.9 $p < 0.005$
Pregnant	24	6.92 ± 0.05 (121)‡	2.29 ± 0.08‡	6.83 ± 0.05 (149)‡ $p < 0.025$	2.33 ± 0.09‡ NS
Postpartum	18	7.09 ± 0.08 (81.6)‡	1.69 ± 0.07	6.73 ± 0.10 (185)‡ $p < 0.001$	1.45 ± 0.10‡ $p < 0.001$

n, number of tissues tested; *NS*, not significant.
*Concentration required to elicit 50% maximum response in nmol/L.
†Statistical significance measured by paired *t* test.
‡$p < 0.01$, evaluated by the Dunnett test.

Table IV. Effect of N^g-monomethyl-L-arginine on sensitivity and responsiveness to phenylephrine in aortic rings of nonpregnant, pregnant, and postpartum rats

		Control		*N^g-monomethyl-L-arginine*	
Group	*n*	*Negative log of concentration producing 50% of maximal response (nmol/L)**	*Maximal response (gm)*	*Negative log of concentration producing 50% of maximal response (nmol/L)**	*Maximal response (%)*
Nonpregnant	50	7.29 ± 0.04 (50.7)	2.04 ± 0.08	8.10 ± 0.09 (8.2) $p < 0.001$†	2.44 ± 0.10 $p < 0.001$
Pregnant	27	7.03 ± 0.05 (92.8)‡	2.16 ± 0.05	7.42 ± 0.04 (37.6)‡ $p < 0.001$	2.47 ± 0.04 $p < 0.001$
Postpartum	24	7.22 ± 0.06 (60.4)‡	1.51 ± 0.10†	8.22 ± 0.13 (5.97)‡ $p < 0.001$	1.74 ± 0.09 $p < 0.005$

n, number of tissues tested.
*Concentration required to elicit 50% maximum response in nmol/L.
†Statistical significance measured by paired *t* test.
‡$p < 0.01$, evaluated by the Dunnett test.

hindered by an endogenous factor whose secretion is blocked by N^g-monomethyl-L-arginine, and although present during pregnancy, this physiologic antagonism seems to have a lesser influence in this situation.

Finally, as EDRF markedly affected the responses of aortic tissues to phenylephrine and could mask the possible influence of prostaglandins on the vascular reactivity to phenylephrine, we tested indomethacin on aortic rings in which EDRF liberation was already blocked. Fig. 5 shows that, in comparison to N^g-monomethyl-L-arginine (0.1 mmol/L) alone *(closed circle)*, when the same aortic rings were under the influence of both N^g-monomethyl-L-arginine (0.1 mmol/L) and indomethacin (10 μmol/L) *(open circle)*, the effect of phenylephrine was not modified. The only significant change observed was a small decrease of maximum response in tissues of pregnant rats by comparison with nonpregnant ones (respectively, 2.57 ± 0.08 vs 2.81 ± 0.06, $p < 0.05$ in the presence of N^g-monomethyl-L-arginine, and 2.49 ± 0.08 vs 2.86 ± 0.06, $p < 0.05$ in the presence of N^g-monomethyl-L-arginine and indomethacin). These results suggest that the liberation of a vasodilating derivative of arachidonic acid through cyclooxygenase does not influence the responses of nonpregnant, pregnant, and post partum aortic rings to phenylephrine.

Comment

In the present study, we investigated one of the possible mechanisms that could be responsible for the blunted response to vasoconstrictors during pregnancy in the rat. The mechanisms proposed during the last few years[15, 17] are (1) autoregulation of receptors for vasoactive agents, (2) increased endogenous liberation of vasodilator agent(s) by the administration of vasoconstrictors, (3) regulation of postreceptor coupling mechanism, and (4) modification of the mechanical properties of the vessel wall. Very recently, Blizard and

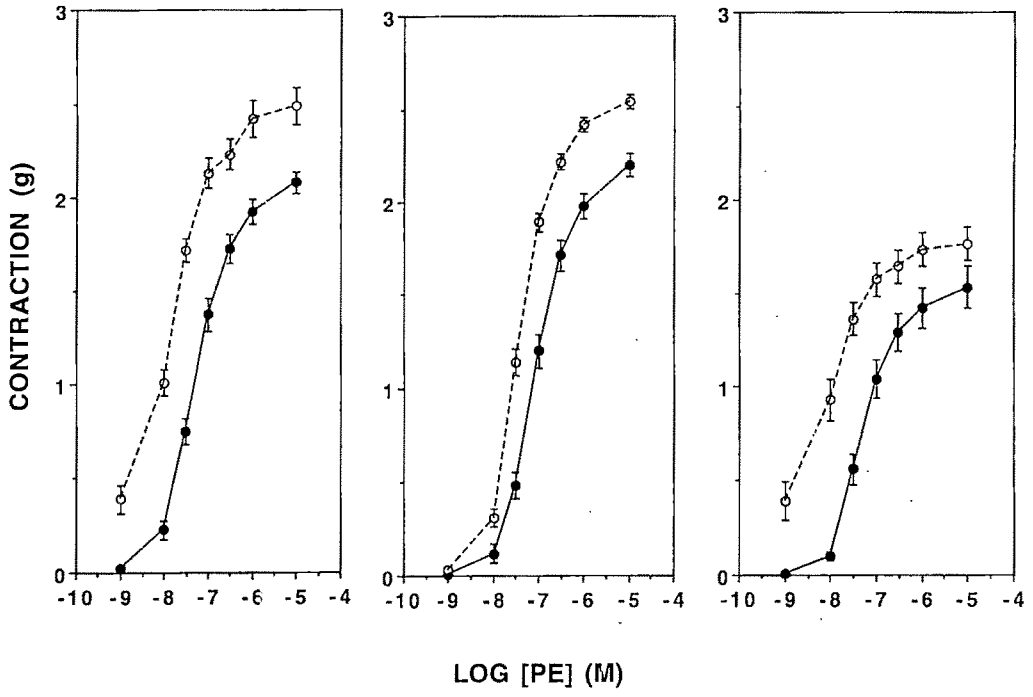


Fig. 4. Concentration-response curves to phenylephrine in absence *(filled circles)* and presence *(open circles)* of N^g-monomethyl-L-arginine (0.1 mmol/L) in aortic rings of nonpregnant *(left)*, pregnant *(middle)*, and post partum *(right)* rats. Symbols and bars represent means ± SE of number of measurements shown in Table IV. Abscissa indicates molar concentration of phenylephrine in logarithm and ordinate, contractile responses in grams.

Folk[18] have proposed that the late gestational decrease in blood pressure in the rat is the result of combined metabolic requirements of maternal and fetoplacental tissues on maternal hemodynamics. This hypothesis, although logically presented and very attractive, remains silent on the pathway mechanisms linking both phenomena. In those experiments we have verified the involvement of the second mechanism proposed above.

The involvement of an increased endogenous influence of vasodilator substance(s) in the blunted response to vasoconstrictors during pregnancy was proposed several years ago[19] and received much attention up to now, but the results obtained have been controversial.[12, 13] The discovery of EDRF[1] furnished an interesting alternative to arachidonic acid derivatives as the putative endogenous agent.

We have observed that the overall reactivity of aortic rings to an endothelium-dependent vasorelaxant such acetylcholine is not modified in tissues from term pregnant and early post partum rats in comparison with nonpregnant controls, although the effects of phenylephrine are decreased in sensitivity under these conditions (Fig. 1). This is in agreement with the results of Teeuw and DeJong,[20] who have shown that the vasodepressor action of acetylcholine on blood pressure is similar in virgin and term pregnant rats. This is also in agreement with recent results obtained in our laboratory,[15] where we have observed that the endothelial lining of the superior mesenteric artery of nonpregnant and pregnant rats similarly influences the responses to adrenergic agents. Weiner et al.[21] reported increased response and efficacy of uterine and carotid artery segments to acetylcholine during pregnancy in the guinea pig. These results were obtained in a protocol similar to ours, except that these authors precontracted their tissues to a lower level of contraction. This has induced a much lower level of contraction, which could be responsible for the higher potency of acetylcholine to relax vessel segments.[22]

EDRF, the tissue mediator of the vasorelaxant effects of acetylcholine and recently proposed to be nitric oxide,[4] activates soluble guanylate cyclase in smooth muscle cells. This vasorelaxant mechanism is shared by nitro vasodilators such as sodium nitroprusside. The action of the latter on aortic rings from pregnant rats is not modified, as reported in the perfused mesenteric vascular bed in vitro.[23] This indicates that the stimulation-relaxation coupling mechanisms for this kind of agents are not altered during pregnancy, at least in such a way as to be responsible for the blunted response to vasoconstrictors. The same is probably true for tissues from post partum rats, although our results (Fig. 2) are consistent with an increase in sensitivity to sodium nitroprusside. This effect may be the consequence of a much lower sensitivity of this group of aortic rings to phenylephrine, resulting in a smaller response (in

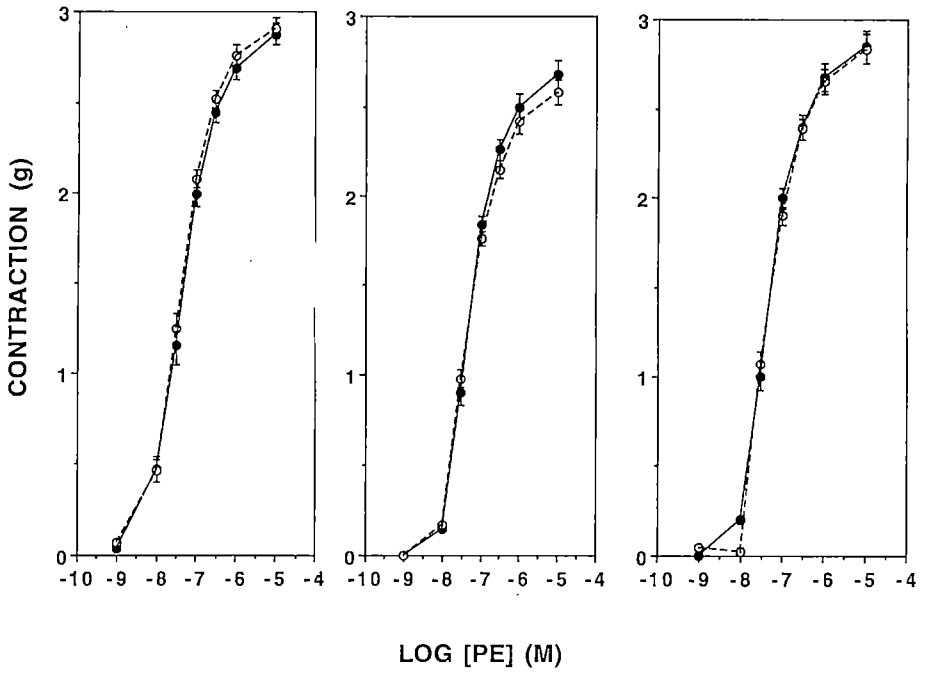


Fig. 5. Concentration-response curves to phenylephrine in the absence *(filled circles)* and presence *(open circles)* of indomethacin (10 μmol/L) in N^g-monomethyl-L-arginine–treated (0.1 mmol/L) aortic rings of nonpregnant *(left)*, pregnant *(middle)*, and post partum *(right)* rats. Symbols and bars represent means ± SE of number of 24 measurements. Abscissa indicates molar concentration of phenylephrine in logarithm and ordinate, contractile responses in grams.

terms of percent maximum response) to 1.0 μmol/L phenylephrine used to precontract the tissues. We have previously demonstrated for atrial natriuretic peptide that a lower level of precontraction on aortic strips results in a significant leftward shift of the vasorelaxant concentration-response curve of this peptide.[22]

The results contained in the present report on the use of N^g-monomethyl-L-arginine also suggest the noninvolvement of EDRF in the resistance to α-adrenergic vasoconstriction during pregnancy. N^g-monomethyl-L-arginine is believed to block the formation of EDRF from tissue L-arginine at the enzyme level.[4] When used to influence the concentration-response curve to phenylephrine (Fig. 4), we observed a substantial increase in overall reactivity to phenylephrine in the three groups of tissues. This is in agreement with our earlier report on superior mesenteric artery rings,[15] in which we showed that the sensitivity of these tissues to phenylephrine and norepinephrine was inversely related to the relative presence of functional endothelium and that of isoproterenol was directly related to it. For the three agents, the just-mentioned relationship was similar in magnitude in tissues from nonpregnant and pregnant rats. This is also in agreement with the results of Umans et al.[24] They reported that bolus intravenous injections of N^g-monomethyl-L-arginine produced similar increases in blood pressure and decreases in heart rate in conscious, instrumented virgin and term pregnant rats. Surprisingly, Jansakul et al.[25] observed a supersensitivity to α-adrenergic agonists on aortic rings at the terminal stage of pregnancy in the rat. This supersensitivity, or more exactly "superresponsiveness," is the opposite of what has been observed many times,[7-9, 15] e.g., a decreased sensitivity to α-adrenergic agents (increased in the concentration producing 50% maximum response) was consistently reported. These authors[25] reported, however, that the removal of endothelium or the presence of oxyhemoglobin significantly increased the response to α-adrenergic agonists as we have reported here.[15] They also showed that the vasorelaxant response to acetylcholine in norepinephrine or phenylephrine-precontracted aortic segments was not modified during pregnancy. This last part is totally in agreement with the results presented here. This is also in agreement with our previous results, which showed a difference of about one log unit on the 50% maximum response between the sensitivity of tissues to phenylephrine with and without a functional endothelium.[15] Furthermore, we must consider that vascular tissue strips, such as aorta, female artery, etc., in which the endothelium has been removed by the preparation procedure are less sensitive to vasoconstrictors when obtained from pregnant animals.[7, 8]

In the present experiments, however, our data suggest that the endothelium has much less influence during pregnancy than in nonpregnant and post partum

periods (Table IV). This is demonstrated by the smaller increases in the log of the concentration producing 50% maximum response values in pregnant than in nonpregnant and post partum tissues. On the contrary, Ahokas et al.[26] recently present an abstract showing an increased blood pressure response to N^g-monomethyl-L-arginine in pregnant spontaneously hypertensive rats, but not in the Wistar-Kyoto normotensive ones.

The data obtained with indomethacin are, however, puzzling. Indeed, in Fig. 3 an inhibition of the contractile response to phenylephrine was observed after the incubation of aortic rings with indomethacin. This is the opposite of what can be expected from the original hypothesis, e.g., of a vasodilator arachidonic acid derivative being responsible for the blunted response to vasoconstrictors. Our results rather indicate that phenylephrine induces the endogenous secretion of a vasoconstrictor factor that helps to contract the tissues. Alternatively, under the influence of indomethacin, the effect of another agent, perhaps through the lipoxygenase pathway, may be unraveled. This last explanation is unlikely because in preliminary experiments we have observed that no such compound is produced by aortic tissue in vitro, in the absence or presence of the same concentration of indomethacin (Falardeau and St-Louis, unpublished data). These explanations are quite speculative at present, however, since the small inhibition with indomethacin does not occur in the presence of N^g-monomethyl-L-arginine (Fig. 5). This observation remains to be fully investigated.

In conclusion, the present results do not support the involvement of arachidonic acid derivatives, by the cyclooxygenase pathway, or of EDRF in the decreased sensitivity to α-adrenergic vasoconstrictors in pregnant and postpartum rats. They also suggest that endogenous EDRF liberation by the vascular wall on the action of vasoconstrictors is much less significant in pregnant than in virgin and postpartum rats. Furthermore, indomethacin is believed to block an endogenous mechanism, helping the action of phenylephrine in aortic rings of the rat, especially in the postpartum period.

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Inhibition of preimplantation mouse embryo development by isoflurane

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OBJECTIVE: Preimplantation mouse embryos were exposed to a commonly used inhalational anesthetic agent, isoflurane, to determine its effects on embryo development.
STUDY DESIGN: Two-cell embryos were exposed at various intervals (5 to 6 hours, 3 to 4 hours, and 0 to 1 hour) before the onset of their first cleavage in vitro. In addition, the effects of 5% isoflurane on four-cell embryos exposed about 2 hours after the first cleavage and morula stage embryos also were examined.
RESULTS: Development to the blastocyst stage was inhibited by 3% and 5% isoflurane ($p < 0.005$) but not by 1.5% isoflurane when two-cell embryos were exposed 3 to 4 hours or 0 to 1 hour before the onset of cleavage. Most of the affected embryos completed cell division and came to a halt at the three- to four-cell stage. The development of embryos exposed to isoflurane at the four-cell or morula stage was unaffected.
CONCLUSIONS: Isoflurane adversely affects subsequent preimplantation development when two-cell mouse embryos are exposed just before the onset of their first cleavage in vitro. (AM J OBSTET GYNECOL 1992;166:693-8.)

Key words: Cleavage, mouse, embryo, isoflurane, anesthesia

An estimated 50,000 pregnant women are exposed to general anesthesia during surgery each year in the United States. In some patients, the operation is performed before the detection of pregnancy; in others, it is necessary in spite of, or because of, a patient's pregnancy. Exposure of operating room personnel to trace concentrations of waste gases may represent another mechanism of anesthetic exposure in pregnancy.[1] This occupational exposure has been suggested to be responsible for a higher incidence of spontaneous abortions among operating room personnel than among various control populations[2] and for a possible increase in the incidence of infertility in female anesthesiologists.[3]

One recently introduced inhalational anesthetic that is widely used is isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether). Isoflurane's low blood solubility and limited extent of metabolism allows rapid induction of anesthesia and prompt clearance from the body after surgery, minimizing the risk of accumulation of toxic metabolites.[4] Data on the outcome of offspring of women exposed to isoflurane during pregnancy is limited.[5] Although no teratogenic effects have been observed in rabbits and rats,[6, 7] increased frequencies of cleft palate, skeletal variations, and fetal growth retardation were observed in pregnant mice exposed to light anesthetic doses of isoflurane.[8] The manufacturer of the anesthetic has reported an increase in the incidence of fetal resorption in pregnant mice exposed to isoflurane at six times the clinical dose used in humans.[9] Little is known about the effect of isoflurane on embryo development before implantation. A preliminary study by Chetkowski and Nass[10] indicated that isoflurane inhibited the development of preimplantation mouse embryos cultured in vitro, whereas nitrous oxide exposure was without effect. In contrast, we recently determined that nitrous oxide also may inhibit mouse embryo cleavage, with the effect being highly dependent on the stage of embryo development.[5] These findings may be of considerable importance in clinical medicine. While general anesthesia is not routinely used for embryo transfer in the United States, anesthesia for transcervical embryo transfer is often used in Great Britain,[11] and transfer of embryos to the fallopian tubes, a procedure in which general anesthesia is widely used, is becoming an increasingly accepted treatment for infertility.[12] The purpose of the present study was to determine the effect of isoflurane on mouse embryos at various times during preimplantation development.

Material and methods

Guidelines approved by the University of Alabama at Birmingham Animal Resources Program for the care and use of animals were followed for these experiments. CB6F1/J female mice (Jackson Laboratory, Bar Harbor, Me.), maintained on 12-hour light/dark cycles, were injected intraperitoneally between 4:00 PM and 10:00 PM with 10 IU pregnant mare's serum gonado-

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Table I. Number of embryos for each experimental condition

	Control	*Isoflurane*
Two-cell stage		
1.5%		
5-6 hours	31	30
3-4 hours	35	35
0-1 hour	44	48
3%		
5-6 hours	32	39
3-4 hours	44	44
0-1 hour	47	41
5%		
5-6 hours	36	41
3-4 hours	36	41
0-1 hour	35	40
Three-to-four-cell stage		
5%	40	51
Morula stage		
5%	35	36

tropin (Organon, Inc., via Diosynth, Inc., Chicago, Ill.) to stimulate follicle development and 48 hours later with 20 to 30 IU of human chorionic gonadotropin (Organon) to initiate ovulation.[13] After this second injection, the females were caged with C57BL/10J males overnight and examined for vaginal plugs the following morning as evidence that mating had occurred. Mated females were killed by cervical dislocation 40 to 44 hours after the human chorionic gonadotropin injection, and their oviducts were removed and transferred to four-well culture dishes. Biggers, Whitten, and Whittingham medium[14] prepared from its individual components in double distilled water and then filtered through 0.22 μm membranes (Millipore, Bedford, Mass.), supplemented with 0.3% bovine serum albumin (Fraction V, Sigma Chemical Co., St. Louis, Mo.), was used for all embryo cultures. Two-cell embryos were flushed from the oviducts with culture medium bovine serum albumin with a blunt, 30-gauge needle. Except for the time during which they were exposed to isoflurane or air (controls), the embryos were maintained in 5% carbon dioxide in air at 37° C.

Exposure of the preimplantation embryos in vitro was accomplished in a plastic, 2 L chamber that was warmed to 37° C in a dry incubator. This chamber was connected to an anesthetic machine set to deliver a total air flow rate of 2.5 L/min. Culture medium was preequilibrated in this chamber for 30 to 60 minutes before the time of exposure, at which time the embryos were transferred to the preequilibrated medium and incubated in the exposure chamber for 30 minutes. Controls were transferred to medium that was preequilibrated in room air at 37° C. No appreciable evaporation of the culture media during the incubation period was detected, as determined by measurement of fluid volumes before and after incubation. After 30 minutes, both exposed and control embryos were transferred back to culture medium in 5% carbon dioxide in air at 37° C and monitored for cleavage and development.

Since induction and maintenance of anesthesia usually require a concentration of 1.5% to 3%,[9] two-cell embryos were exposed to 1.5%, 3%, and 5% isoflurane at intervals approximately 5 to 6 hours, 3 to 4 hours, and 0 to 1 hour before the expected onset of cleavage at approximately 12:00 PM to 1:00 AM on the morning after the embryos were collected (data not shown). Usually several embryos had already cleaved before the final exposure. These embryos were removed from these experiments or treated as four-cell embryos. Four-cell embryos were treated only with 5% isoflurane within 2 hours after cleavage, and morula stage embryos were treated with 5% isoflurane before blastocoel formation.

Development of the embryos was observed periodically and compared with the development of the controls. Embryos were classified as two-cell, three- to four-cell, five- to eight-cell, morula stage, and blastocyst stage embryos for purposes of monitoring in vitro cleavage. Abnormal morphology at any stage was noted. The percentage of embryos to cleave to the blastocyst stage after 72 hours of culture was determined.

Experiments were performed at least three times to ensure reproducibility, with between 30 and 51 total embryos used for each condition (Table I). For each experiment embryos collected from mice were pooled and then randomly assigned to an experimental condition. Separate control groups were used for each anesthetic concentration tested at each time point to minimize any possible variation in embryo quality among different experiments. Comparisons between treated and control groups of embryos were performed with χ^2 analysis.

Results

When two-cell embryos were exposed to 5% isoflurane before the onset of cleavage in vitro, significant effects were observed (Fig. 1). Whereas 75.6% of embryos exposed 5 to 6 hours before onset developed into blastocysts compared with 86.1% for the controls, only 19.5% and 35% of those exposed 3 to 4 hours and 0 to 1 hour before onset, respectively, developed to that stage, compared with 75% ($p < 0.005$) and 68.6% ($p < 0.025$) for the controls. Among the embryos that continued to develop in the treated groups, an increase in cell fragmentation was apparent, and blastocoel size was observed to be smaller than in the control groups (Fig. 2).

Similar effects were observed when embryos were treated with 3% isoflurane. In those exposed 3 to 4 hours before onset, only 36.4% developed into blastocysts, compared with 77.2% for the controls

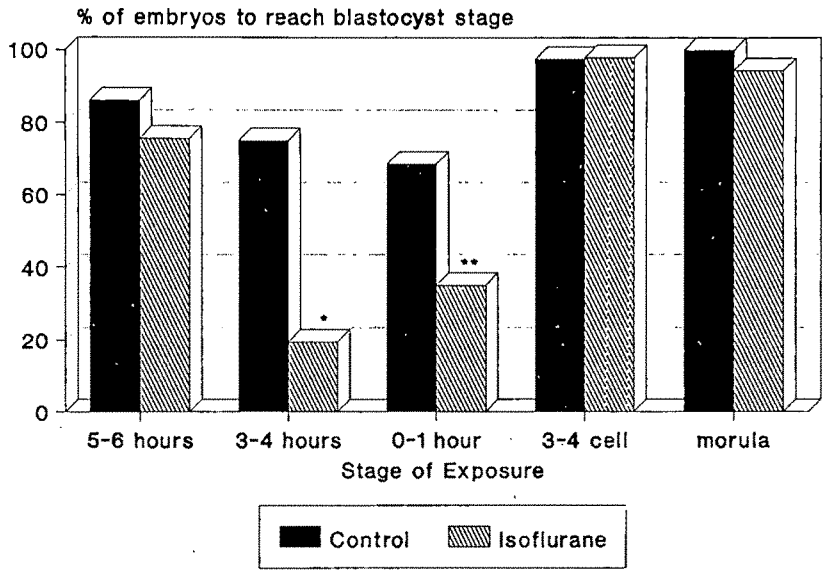


Fig. 1. Developmental outcome of embryos exposed in vitro to 5% isoflurane for 30 minutes at various times in relation to expected onset of the first cleavage in vitro. *Asterisk*, $p < 0.005$; *double asterisk*, $p < 0.025$.

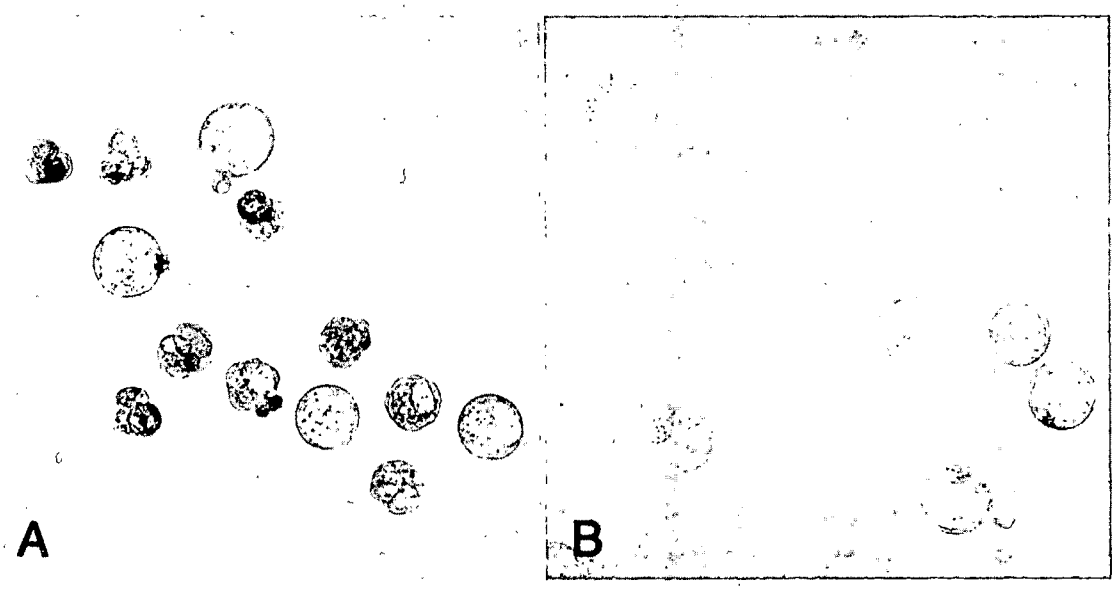


Fig. 2. Preimplantation development of mouse embryos after 30 minutes' exposure in vitro to 5% isoflurane **(A)** or air **(B)** at 3 to 4 hours before onset of first cleavage in vitro. Note halt of development at earlier stages and smaller blastocoel cavities in treated group. (Original magnification ×150.)

($p < 0.005$), whereas in those exposed 0 to 1 hour before onset, 31.7% developed, compared with 87.2% for the controls ($p < 0.005$) (Fig. 3). These effects were not observed when the exposure level was reduced to 1.5% (data not shown). Even when embryos were exposed 0 to 1 hour before the onset of cleavage, 79.2% of treated embryos developed into blastocysts, compared with 79.5% of the controls. No effects on subsequent development were observed when four-cell and morula-stage embryos were treated with 5% isoflurane (Fig. 1).

In those groups in which development to the blastocyst stage was affected by isoflurane, periodic assessment of the extent of cleavage was undertaken to determine the point at which development was halted. Of the two-cell embryos exposed to 5% isoflurane 0 to 1 hour before onset, 85% cleaved to the three- to four-cell stage, but only 52.5% continued to the five- to eight-

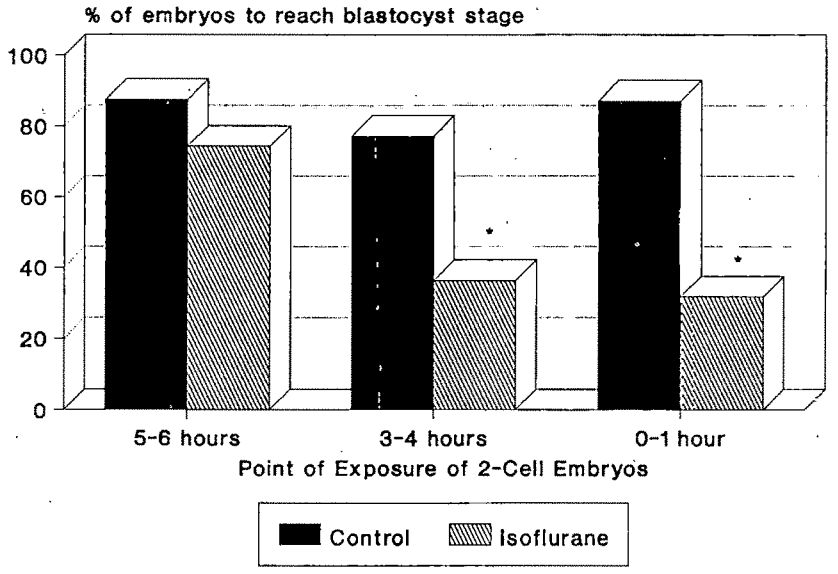


Fig. 3. Developmental outcome of embryos exposed in vitro to 3% isoflurane for 30 minutes at various times in relation to expected onset of the first cleavage in vitro. *Asterisk*, $p < 0.005$.

cell stage (Fig. 4). In those exposed to 3% at approximately the same time point, 80.5% cleaved to the three- to four-cell stage, but only 41.5% continued developing (Fig. 5). In general, the proportion of treated embryos to undergo the first in vitro cleavage was comparable to that of the control group, with the deleterious effect of isoflurane on embryo development becoming more apparent as the posttreatment incubation progressed.

Comment

In this study, isoflurane was shown to affect the development of preimplantation mouse embryos exposed at the late two-cell stage. When two-cell embryos were exposed to 3% and 5% isoflurane within 3 to 4 hours before the onset of cleavage, the development in a significant number of embryos was halted after the first cleavage in vitro. However, the subsequent cleavage of four-cell embryos exposed within 2 hours after this first cleavage was not affected by isoflurane exposure. Because approximately 90% of two-cell embryos normally progress to the four-cell stage, differences in embryo quality between different developmental stages would not explain these results. We feel that the effects are probably due to differences in sensitivity at various points within the cell cycle.

Cytotoxic agents prevent cell division from occurring in actively dividing cells by disrupting key metabolic functions, energy metabolism, or the mitotic spindle. For example, aphidicolin, which inhibits deoxyribonucleic acid (DNA) synthesis by specifically inhibiting DNA polymerase-α,[15] and cycloheximide, which primarily inhibits protein synthesis[16]; both inhibit cell proliferation. Other agents such as colchicine, which binds to tubulin, inhibit polymerization of the mitotic spindle and thus block mitosis without directly affecting cell metabolism.[17] The cytotoxic effects of anesthetic agents were first recognized in 1878, when Bernard reported that ether vapors retarded the growth of plant seedlings.[18] Some anesthetic agents apparently interrupt mitosis by arresting cells in metaphase, possibly by the reversible dispersal of labile microtubules and depolymerization of the mitotic spindle during M phase.[19] Inhibition of DNA synthesis during the S phase,[20] interference with prometaphase movement,[21] and prolongation of the G_2 postsynthetic phase[22] have also been suggested to be associated with various anesthetic agents. Thus the effects of isoflurane observed in this study could be caused by a variety of specific actions alone or in combination.

Unlike most other cell types, in which cell mass doubles before cleavage, preimplantation embryos undergo reductive cleavage and require relatively little preparation for cytokinesis (cytoplasmic division). For this reason, most energy expenditure and synthetic activity is directed toward DNA synthesis and mitosis. DNA replication (S phase) lasts 6 to 7 hours, whereas the G_1 phase, if it exists at all, appears to last only 1 to 2 hours. The G_2 phase, which lasts 12 to 15 hours in two-cell embryos, is reduced sharply in subsequent cycles.[23] The results of the present study suggest that the effect of isoflurane may be limited to the late G_2 or M phases in mouse preimplantation embryos. It is possible that the increased cell-cycle asynchronicity that occurs with continued preimplantation embryo cleavage limits the deleterious effect of isoflurane exposure at later stages of development.

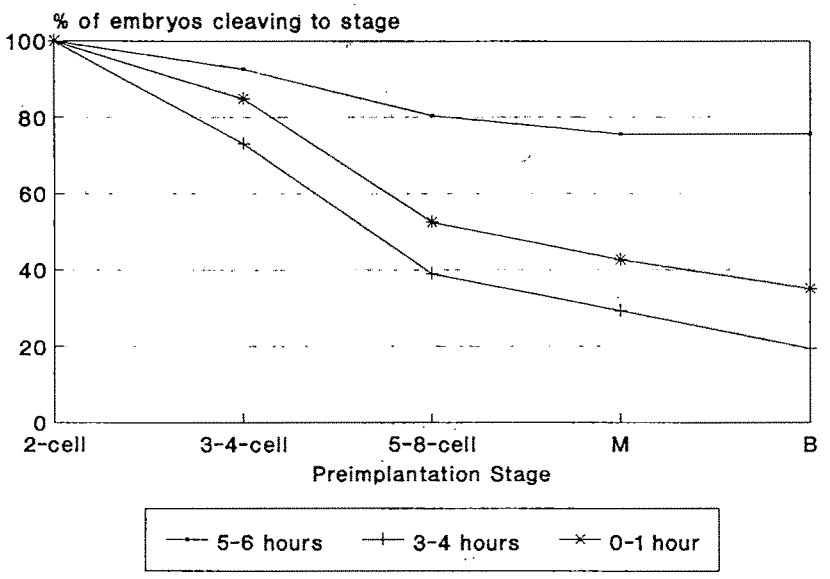


Fig. 4. Cleavage of embryos after exposure to 5% isoflurane at various times before expected onset of first cleavage in vitro.

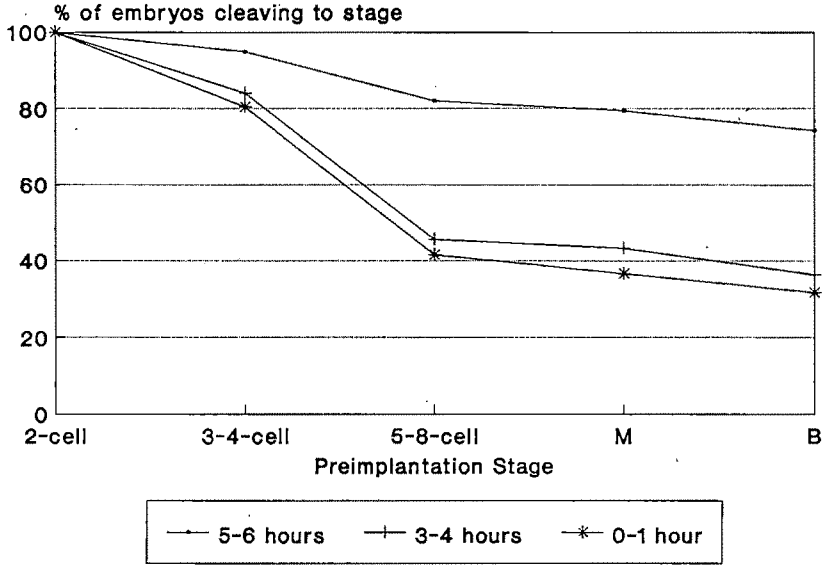


Fig. 5. Cleavage of embryos after exposure to 3% isoflurane at various times before expected onset of first cleavage in vitro.

We previously have reported that nitrous oxide exhibits a comparable cytotoxic effect in two-cell mouse embryos exposed just before cleavage.[5] However, in contrast to the effect of isoflurane, nitrous oxide treatment significantly inhibited any subsequent cell division, whereas the effect of isoflurane exposure did not become apparent until after the first in vitro cleavage. This suggests that these two anesthetic agents may exert their effects through different mechanisms. Further studies will be needed to characterize the nature of these effects.

The results of this and our previous study[5] demonstrate that the effects of anesthetics on preimplantation embryo cleavage vary markedly with stage of development and point of exposure of the embryos. The findings of previous studies indicating the absence of deleterious effects of nitrous oxide exposure may have been due to exposure before fertilization[24] or failure to rigorously control for the timing of the developmental stages at which embryos were exposed.[10] In contrast to Chetkowski and Nass,[10] we were unable to demonstrate an effect of isoflurane at a concentration of 1.5%.

Whether this was due to differences between the embryos of different mouse strains, differences in the anesthetic mixture, or some other reason is unclear. In their studies, embryos were exposed to anesthetics in phosphate-buffered saline and then transferred to Ham's F-10 medium (without protein supplementation), whereas our experiments used Biggers, Whitten, and Whittingham medium with bovine serum albumin throughout the exposure and culture period. Differences in electrolyte concentrations in solutions of equal osmolarity have only minor effects on the solubility of isoflurane,[25] and thus it is unlikely that media composition per se would account for the observed differences. The 30-minute incubation of the bicarbonate-based culture medium solution in air/isoflurane could cause a significant fall in pH, but one would expect that any pH changes due to decreased carbon dioxide levels in the media during exposure would have affected the treated and control groups equally. However, we have previously noted a beneficial effect of bovine serum albumin on mouse embryo survival, and it is possible that the more favorable culture conditions in our study might have attenuated the deleterious effect of isoflurane. Also, albumin has been reported to interact with halogenated anesthetics,[26] and an effect of protein supplementation on the bioavailability of isoflurane cannot be ruled out. Nevertheless, the concentration of isoflurane at which we noted significant effects is within the range used in human anesthesia.[9] Interestingly, Fishel et al.[11] suggested that the choice of anesthesia for transfer of cleaving human embryos could be a factor in in vitro fertilization pregnancy rates. We suggest that caution be exercised with respect to the choice of anesthesia for clinical procedures involving the transfer of cleaving embryos.

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Venous drainage of the human uterus: Respiratory gas studies in normal and fetal growth-retarded pregnancies

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OBJECTIVE: To determine respiratory gas relationships between the uterine veins and umbilical vein in normal and pregnancies complicated by intrauterine growth retardation.

STUDY DESIGN: Respiratory gases were measured in both uterine veins and the umbilical vein in eight normal and 13 pregnancies with intrauterine growth retardation.

RESULTS: No significant differences were found in the placental versus nonplacental uterine veins. There was a significant correlation for umbilical and uterine venous values of Po_2 ($p < 0.002$) and Pco_2 ($p < 0.004$) in appropriate-for-gestational-age pregnancies, umbilical venous Po_2 was always less than uterine venous Po_2, and Pco_2 always greater than uterine. The transplacental gradient was significantly higher in intrauterine growth retarded than appropriate-for-gestational-age pregnancies for both Po_2 and Pco_2. There was a lower uterine oxygen extraction in intrauterine growth retarded pregnancies ($p < 0.05$).

CONCLUSION: There is no consistent relationship between placental venous drainage in each uterine vein and placental location. The human placenta simulates a relatively inefficient venous equilibrator and the larger transplacental gradients in intrauterine growth retarded pregnancies may reflect differences in both perfusion pattern and placental structure. (Am J Obstet Gynecol 1992;166:699-706.)

Key words: Respiratory gases, uterine venous drainage, pregnant uterus, transplacental gradient, intrauterine growth retardation

The physiologic characteristics of the venous drainage of the human uterus are not well known, e.g., the proportion of the total venous drainage of the uterus representing drainage of the placenta versus myometrial and endometrial drainage has yet to be defined. In addition, it is not known whether the proportion of placental to nonplacental venous drainage in each uterine vein differs as a function of placental location. To study uterine metabolism in man, it is essential to obtain venous samples representative of the mixed placental and nonplacental drainage. In sheep carrying single fetuses with placental cotyledons in both uterine horns, venous samples drawn simultaneously from the right and the left uterine veins have virtually identical composition and thus either uterine vein may be sampled for determination of substrate fluxes.[1] Conversely, a remarkable variability in the venous drainage of the pregnant uterus has been demonstrated in the pregnant monkey, with large and unequal arteriovenous differences in the two veins.[2, 3] The effect of placental location on uterine artery velocimetry has been studied in human pregnancies, and reduced vascular resistance has been reported for the uterine artery ipsilateral to the placenta.[4] These findings suggest that there may be consistent differences in respiratory gas concentrations between the right and left sides of the uterine venous drainage as function of placental location.

The study of the respiratory gas pattern of the venous drainage of the human uterus is also important in providing insight into the perfusion pattern of the human placental circulation. In species with a hemochorial, labyrinthine placenta, such as guinea pigs[5] and rabbits,[6] the maternal and fetal placental circulations simulate a countercurrent exchange system. The horse, which has a diffuse epitheliochorial placenta, also functions as a countercurrent exchanger.[7] In other mammals with an epitheliochorial placenta, such as the sheep, the two circulations form an exchange system, which has been called a "venous equilibrator," with a low degree of effectiveness.[8, 9]

The effectiveness of the human placenta has not been clearly defined. However, previous studies on placental oxygen transfer have suggested that the venous equilibration model may be applicable to the human placenta.[10, 11]

The purposes of the present study were to (1) study

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respiratory gas concentrations in both uterine veins in relation to placental location; (2) compare uterine venous gas tensions with those obtained from the umbilical vein at delivery, to evaluate the perfusion characteristics of the placental circulation (i.e., venous equilibrator vs countercurrent exchanger); and (3) determine whether pregnancies complicated by fetal growth retardation present differences in uterine venous respiratory gas concentrations consistent with changes in uterine perfusion relative to the metabolic demands of the conceptus.

Patients and methods

Informed consent was obtained from all patients, and the procedures were approved by the Institute Board of the Department of Obstetrics and Gynecology of the University of Milan, which has the responsibility for the evaluation of all clinical studies.

Clinical studies. Twenty-one patients undergoing elective cesarean section were studied at 35 to 40 weeks' gestation. Gestational age was calculated from the last menstrual period and confirmed by serial ultrasonographic examinations. Eight of these pregnancies were normal, terminating in the delivery of fetuses whose birth weights were appropriate for gestational age (AGA) using standards developed for the Italian population.[12] The indications for cesarean section in these patients were either repeat cesarean section or breech presentation. Thirteen pregnancies were complicated by intrauterine fetal growth retardation (IUGR), and fetuses were small for gestational age at birth. IUGR was determined by serial ultrasonographic measurements and confirmed at birth by fetal birth weights <2nd percentile according to Italian Standards.[12] Cesarean sections were performed with the patients in the left lateral decubitus position after an overnight fast. Intravenous thiopental sodium was used for induction of anesthesia, and general anesthesia was maintained by ventilation with a concentration of 60% nitrous oxide and 40% oxygen. Blood pressure was carefully monitored and no episodes of significant hypotension were detected. The placental location was determined by ultrasonography before the beginning of the surgical procedure. In the 21 patients studied, the placenta was lateral in 19 cases and central in two cases.

To sample the uterine veins, the vesicouterine serosa was incised and the bladder gently separated by finger dissection from the underlying uterine wall. The main branches of the uterine venous plexuses were visualized. Both right and left uterine veins were sampled with a 25-gauge needle connected to a heparinized syringe immediately before the transverse uterine incision of the inferior segment. The time interval from induction of anesthesia to uterine venous sampling was <5 minutes in all cases. At the same time, arterial blood was sampled from the maternal radial artery. The additional time required for the entire procedure of preparing the veins and sampling the blood was never >1 minute. No complications were observed during and after surgery. Umbilical venous and arterial blood samples were withdrawn from a doubly clamped segment of the cord immediately after fetal extraction.

Analytic procedures. Blood samples were treated as anaerobically as possible and kept on ice until analyses were completed, usually within 10 minutes. pH, P_{O_2}, and P_{CO_2} were measured with an ABL 330 Radiometer (Denmark). Hemoglobin concentration and oxygen saturation were measured in duplicate with a Radiometer OSM_2 Analyzer. As a check on the validity of the anaerobic collection of blood samples, in vivo dissociation curves were constructed for both the fetal and maternal data in all patients.

Calculations. Oxygen content was calculated according to the equation

$$O_2 \text{ cont (mmol/L)} = \text{hemoglobin (gm/L)} \times \text{oxygen saturation} \times 0.05982 \text{ (mmol/gm)}$$

and the percent of uterine oxygen extraction was calculated as

$$\text{Percent } O_2 \text{ extr} = [(A\text{-}V)\ O_2 \text{ cont (mmol/L)} / (A)\ O_2 \text{ cont (mmol/L)} \times 100$$

where *O_2 cont* is oxygen content, *O_2 extr* is oxygen extraction, *A* is maternal radial artery, and *V* is right and left uterine veins.

Statistics. Paired Student *t* test was used to calculate the significance of the difference between placental and nonplacental uterine veins (Table I). Comparison between values obtained in AGA and IUGR pregnancies were made with Mann-Whitney tests (Table II). Regression analyses were done by the least-squared method. Data were expressed as mean ± SEM.

Results

The data from 21 patients (13 IUGR and eight AGA) were used for the analysis below. The clinical data for all these patients are summarized in Table III.

Placental and nonplacental uterine oxygenation. Table I presents P_{O_2}, P_{CO_2}, oxygen saturation, oxygen content, and percent uterine oxygen extraction in the placental and nonplacental uterine veins of both AGA and IUGR pregnancies. No significant differences were observed between placental and nonplacental values for all parameters studied. Note that the average values reported in Table I refer only to those pregnancies that presented a lateral (right or left) placental localization by ultrasongraphy. Central placental pregnancies are omitted (two IUGR pregnancies).

Fig. 1 presents the relationship between placental and nonplacental uterine venous oxygen saturations

Table I. Comparison between placental and nonplacental values in AGA and IUGR pregnancies

	Placental value	*p value*	*Nonplacental value*
AGA			
P_{O_2} (mm Hg)	48.8 ± 4.7	NS	49.4 ± 2.0
P_{CO_2} (mm Hg)	37.0 ± 2.1	NS	35.8 ± 1.6
Oxygen saturation (%)	71.9 ± 5.3	NS	74.6 ± 2.6
Oxygen content (mmol/L)	4.72 ± 0.23	NS	5.01 ± 0.32
Coefficient oxygen extraction (%)	26.8 ± 5.4	NS	24.2 ± 2.8
IUGR			
P_{O_2} (mm Hg)	57.8 ± 5.3	NS	56.4 ± 4.0
P_{CO_2} (mm Hg)	33.7 ± 1.4	NS	33.8 ± 1.8
Oxygen saturation (%)	82.4 ± 4.1	NS	82.3 ± 3.4
Oxygen content (%)	6.36 ± 0.39	NS	6.35 ± 0.35
Coefficient oxygen extraction (%)	16.5 ± 4.3	NS	16.8 ± 3.6

NS, nonsignificant. Data are expressed as mean ± SE.

Table II. Comparison of uterine oxygenation in AGA and IUGR pregnancies

	AGA	*p value*	*IUGR*
Uterine venous			
P_{O_2} (mm Hg)	44.2 ± 2.8	NS	52.1 ± 3.2
P_{CO_2} (mm Hg)	37.8 ± 1.6	NS	34.4 ± 1.4
Oxygen saturation (%)	66.8 ± 3.6	*	79.1 ± 3.7
Oxygen content (mmol/L)	4.46 ± 0.26	†	5.92 ± 0.36
Maternal arterial			
Oxygen content (mmol/L)	6.58 ± 0.31	*	7.38 ± 0.24
Hemoglobin (gm/dl)	11.2 ± 0.52	*	12.5 ± 0.4
Uterine maternal radial artery-right and left uterine veins			
Oxygen content (mmol/L)	2.14 ± 0.32	*	1.46 ± 0.29
Uterine coefficient oxygen extraction (%)	32.1 ± 3.8	*	19.9 ± 3.8
Fetal venous			
P_{O_2} (mm Hg)	27.6 ± 2.6	NS	23.8 ± 1.7
Oxygen content (mmol/L)	5.02 ± 0.52	NS	4.37 ± 0.40
Fetal arterial			
Oxygen content (mmol/L)	2.0 ± 0.45	NS	2.06 ± 0.32
Hemoglobin (gm/dl)	15.3 ± 0.36	*	17.0 ± 0.49
Umbilical maternal radial artery-right and left uterine veins			
Oxygen content (mmol/L)	3.02 ± 0.31	NS	2.42 ± 0.38

*$p < 0.05$.
†$p < 0.01$.
NS, not significant. Data are expressed as mean ± SE.

with an identity line shown for comparison. Note that there were no systematic differences between placental and nonplacental values; the data were characterized by a great variability among patients for the placental-nonplacental differences. A similar relationship between placental and nonplacental values was found for the other parameters under study. There were no variables measured in which there were systematic differences between placental and nonplacental values.

Uterine-umbilical respiratory gas exchange. To better understand the relationship between the umbilical and uterine venous samples for respiratory gases, the data were analyzed in two different ways. First, to obtain an estimate of mixed uterine venous drainage, the mean of the placental and nonplacental uterine venous values was calculated and used to study the relationship between uterine venous and umbilical venous values. Second, the data were analyzed using the uterine venous values that were least oxygenated in each case, regardless of the anatomic location of the placenta. The reason for this is that such an analysis provides a minimal estimate of the P_{O_2} and P_{CO_2} gradients between the two streams. Such an analysis would tend to maximize an estimate of the efficiency of the human placenta. Figs. 2 and 3 present the data for both AGA and IUGR patients. There were no significant relationships in the IUGR patients; however, there were highly significant relationships for the normal pregnancies. The regression analyses are presented in the legends. Note that the mean P_{O_2} difference in these AGA patients was 16.7 ± 1.2 torr, and the mean P_{CO_2} difference was −7.1 ± 0.4 torr. Cases with central placental location

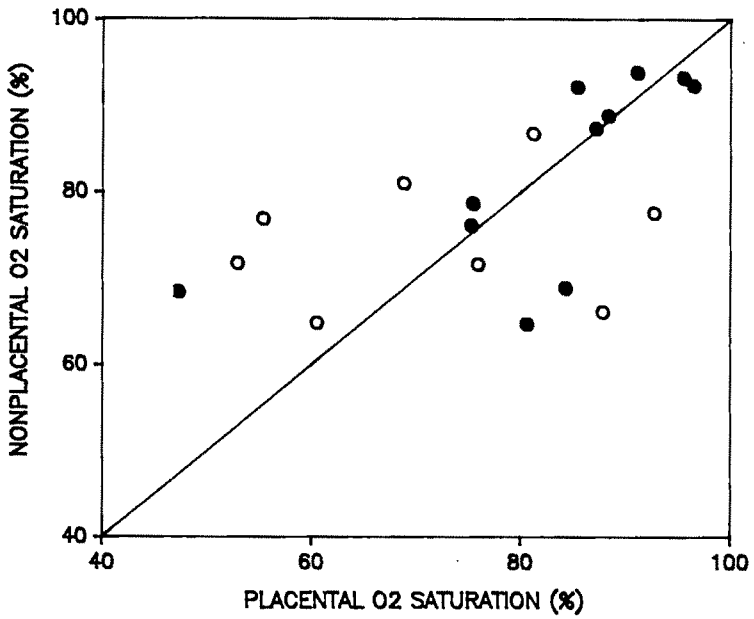


Fig. 1. Comparison of oxygen saturations in placental and nonplacental uterine veins of AGA (○) and IUGR (●) pregnancies. Identity line is included in figure for reference.

Table III. Clinical information for the AGA and IUGR pregnancies

Patient	*Maternal age (yr)*	*Gestational age (wk)*	*Fetal weight (gm)*	*Placental weight (gm)*	*Placental location*
AGA					
1	35	39	3000	427	Right
2	31	38	3880	425	Right
3	28	35	3000	427	Right
4	30	39	2980	420	Right
5	29	39	3000	368	Right
6	24	39	3000	400	Right
7	25	40	3600	335	Right
8	31	39	3640	690	Left
Mean	29.1 ± 1.2	38.5 ± 0.5	3262.5 ± 133.2	436.5 ± 38.1	
IUGR					
1	43	37	2100	277	Right
2	29	35	1550	227	Right
3	28	36	2000	272	Left
4	19	40	2340	360	Left
5	30	35	1700	208	Right
6	21	35	1600	270	Right
7	28	36	1700	190	Left
8	26	35	1800	270	Right
9	25	37	2070	220	Right
10	31	36	1830	470	Right
11	30	39	2280	390	Right
12	33	38	2150	280	Control
13	28	35	1800	250	Control
Mean	28.5 ± 1.6	36.5 ± 0.5	1916.9 ± 71.3	283.4 ± 22.0	

Data are expressed as mean ± SE.

were included in this analysis. P_{O_2} was always lower in the umbilical vein than in the mean uterine vein, and P_{CO_2} was always higher in umbilical venous than in uterine venous samples.

Uterine oxygen extraction and placental respiratory gas gradients in AGA and IUGR pregnancies. Table II compares values found in AGA and IUGR pregnancies for respiratory gases in the uterine venous drainage. For each patient the uterine venous values with the lowest oxygenation were compared as most representative of placental drainage, regardless of placental location. However, none of the differences between AGA and IUGR pregnancies are altered if mean uterine venous values are compared. As expected,

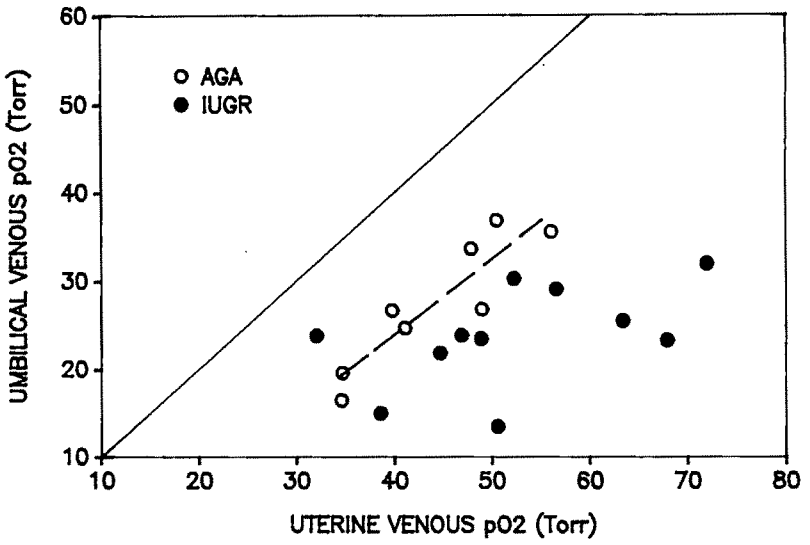


Fig. 2. Relationship between uterine venous and umbilical venous P_{O_2} in AGA (o) and IUGR (●) pregnancies. In this and subsequent figures, least oxygenated value for uterine vein was used. For AGA pregnancies regression line is presented ($y = -10.39 + 0.86x; r = 0.90, p = 0.002$). For IUGR pregnancies relationship is not significant. *Solid line* represents identity line.

IUGR fetuses had significantly higher hemoglobin concentrations. Oxygen content and saturation were significantly higher in the uterine vein of IUGR pregnancies ($p < 0.01$ and $p < 0.05$, respectively). The uterine arteriovenous difference for oxygen content was significantly greater in AGA pregnancies ($p < 0.05$), and the percent uterine oxygen extraction was significantly lower ($p < 0.05$) in IUGR pregnancies. The transplacental P_{O_2} and P_{CO_2} differences were calculated for the two groups of patients. Fig. 4 presents the mean transplacental difference between uterine vein and umbilical vein for P_{O_2} and P_{CO_2} in AGA and IUGR pregnancies. The gradient is significantly higher in IUGR pregnancies for both P_{O_2} ($p < 0.01$) and P_{CO_2} ($p < 0.001$).

Comment

The present study addresses the question of the pattern of respiratory gas exchange across the human placenta. Our data clearly demonstrate that in all cases the umbilical venous P_{O_2} is lower than the uterine venous P_{O_2} and, conversely, that umbilical venous P_{CO_2} is always higher than that in the uterine venous blood. Such a pattern of exchange is consistent with what has been termed a venous equilibrator. A variety of different perfusion patterns within the hemochorial placenta could lead to these results, including venous shunting (anatomic or diffusional) or uneven flow ratios. The current studies cannot discriminate between these possibilities. The hemochorial placenta of the human is different in this regard from the hemochorial placentas of guinea pigs and rabbits where the respiratory gas pattern is consistent with a countercurrent exchanger, i.e., umbilical venous P_{O_2} exceeds uterine venous P_{O_2}.[5, 6, 13] The present data for the human placenta are similar to data collected in the rhesus monkey. Two different in vivo studies have confirmed that the rhesus monkey placenta simulates a venous equilibrator.[2, 3] One hypothesis proposed by Bartels and Moll[14] is that the human placenta may function as a multivillous model. Such a model would be much more efficient than a venous exchanger, because umbilical venous P_{O_2} may be equal to or exceed maternal venous P_{O_2}.

By analyzing the uterine venous P_{O_2} that was lowest in each case, we sought to minimize our estimated uterine vein-umbilical vein P_{O_2} difference. In spite of this, the P_{O_2} difference was quite large (16.7 torr), comparable to that found in sheep (approximately 20 torr).

Another estimate of the minimal uterine venous-umbilical venous P_{O_2} can be obtained by using our data for uterine venous P_{O_2} in normal pregnancies (44.2 torr) compared with umbilical venous P_{O_2} measured at the same gestational ages from samples obtained at cordocentesis, without the stress of cesarean section (32.3 torr). Such analysis yields a P_{O_2} difference of 11.9 torr. Thus both attempts to minimize the P_{O_2} difference give estimates between 10 and 16 torr. Therefore, these data do not lend support to a multivillous model but rather favor a model of a venous equilibrator, similar to that found in the rhesus monkey and in the sheep.

A second point addressed by the present study concerns the interpretation of uterine venous metabolic data in pregnant women. One might have supposed that placental drainage would occur principally into the

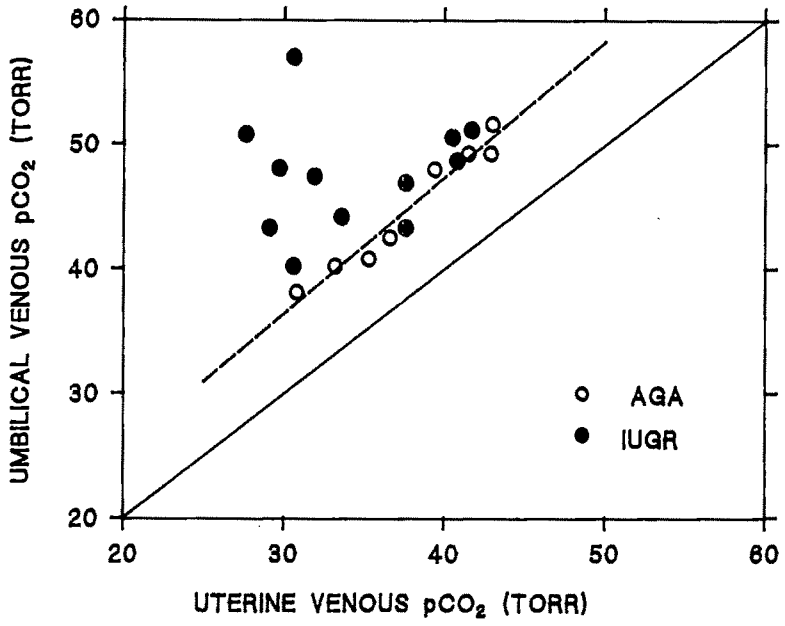


Fig. 3. Relationship between uterine venous and umbilical venous P_{CO_2} in AGA (○) and IUGR (●) pregnancies. For AGA pregnancies regression line is presented ($y = 3.61 + 1.09x$; $r = 0.98$; $p < 0.001$). For IUGR pregnancies relationship is not significant. *Solid line* represents identity line.

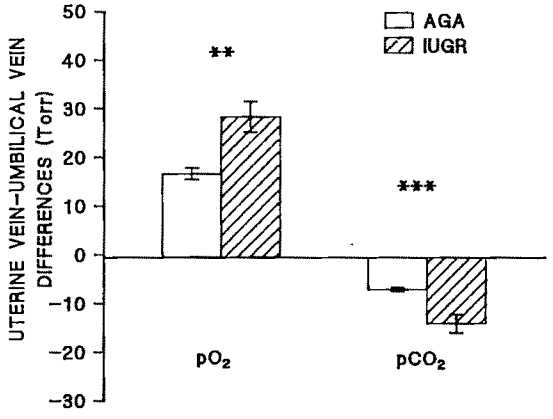


Fig. 4. Comparison of uterine venous-umbilical venous P_{O_2} and P_{CO_2} differences in AGA (□) and IUGR (▨) pregnancies. *Double asterisk*, $p < 0.01$; *Triple asterisk*, $p < 0.001$.

uterine vein on the same side of the uterus as the placenta. The present data clearly demonstrate that this is not a consistent relationship. There is a great deal of variability from one pregnancy to the next, a variability which was also found for the venous drainage of the rhesus monkey uterus.[2, 3]

The study focuses attention on a new question of considerable clinical relevance. The present data demonstrate that the placental venous drainage is not distributed evenly between the two uterine veins. It is possible that in some pregnancies the bulk of the venous drainage of the placenta occurs through the venous drainage on one side of the uterus. In these cases, occlusion of that uterine vein, which could occur from positional changes in the mother, would markedly affect placental transport. This has been demonstrated in the two studies in rhesus monkeys and is a question which may now be open to investigation in pregnant women, using improving techniques for ultrasonographic visualization of vessels and Doppler velocimetry.

The data in the present study were collected at the time of elective cesarean section. Inevitably, there are changes in uterine tone and in placental perfusion induced by manipulation of the uterus or by the uterine incision. Such changes would tend to increase the P_{O_2} difference between the two venous circulations, particularly at high P_{O_2}, reducing the slope of the relationship between umbilical venous and uterine venous P_{O_2} (Fig. 2). The major point demonstrated in Fig. 2 is that umbilical venous P_{O_2} is always lower than uterine venous P_{O_2} at all levels studied.

It is useful to compare these data with other species. In the present study the coefficient of oxygen extraction for normal pregnancies is 32.1% ± 3.8%, similar to the value of 24.2% ± 1.1% obtained in pregnant sheep[15] and much lower than the value of 60.6% ± 0.3% found for pregnant rabbits[16] and 71.7% ± 0.5% for guinea pigs.[17] These differences are reflected in much lower uterine venous P_{O_2} values in rabbits, 29.3 ± 0.1 torr than in sheep, 50 ± 1 torr[16] and also lower than our results for human pregnancies, 44.2 ± 3 torr. These data suggest that the rate of perfusion of the human uterus per millimole per liter of

oxygen used is quite large compared to small mammals such as the guinea pig and rabbit. If one accepts the estimates of uterine blood flow in man of approximately 600 ml/min[18] and the average value for the lowest uterine oxygen saturation measured in the present study (66.8%), the uterine oxygen consumption is in the order of 1.5 mmol/L/min. This is not dissimilar from the measured oxygen consumption in pregnant sheep in late gestation (1.65 mmol/L/min).[8]

Comparison of IUGR and normal pregnancies. In the present study there are two striking observations that come out of a comparison of the data for IUGR and normal pregnancies. The first is that uterine venous oxygen content is higher and the coefficient of oxygen extraction is less in IUGR pregnancies than in normal pregnancies (Table II). There is no evidence from these data that the uterus is underperfused relative to oxygen demands in IUGR pregnancies. However, this does not exclude the possibility that a reduction in uterine blood flow earlier in pregnancies may alter the growth and maturation of the placenta and fetus. Doppler velocimetry studies have provided evidence for increased resistance in uterine vessels of IUGR pregnancies associated with maternal hypertension.[19] But the present data make clear that any reductions in uterine flow in IUGR pregnancies are at least proportional to the decreased oxygen requirements of the IUGR uterus.

The second finding of note in the present study is that of an increased transplacental P_{O_2} and P_{CO_2} difference in IUGR patients. This finding of itself has two important physiologic implications. The first is that given the higher oxygen concentration in uterine venous blood of IUGR patients, the increased transplacental respiratory gas gradients demonstrate a decreased placental permeability for IUGR placentas. Presumably this is a reflection of a markedly decreased surface area in these small placentas. A number of histologic changes have been described in IUGR placentas, which would effectively decrease the diffusing capacity or increase shunting of uneven perfusion.[20, 21] More recently, such histopathologic changes in the villi of IUGR placentas have been correlated with abnormal Doppler velocimetry findings.[22-24]

Another potential consequence of an increased transplacental respiratory gas gradient is that umbilical venous P_{O_2} may be decreased in these pregnancies. Data collected by our group[25] and by Nicolaides et al.[26] at cordocentesis have shown that the majority of IUGR fetuses have normal umbilical venous P_{O_2}. For those IUGR fetuses who develop hypoxia, our data help clarify the reasons for such findings, namely the reasons reside within the placenta and its fetal perfusion and are not a function of decreased uterine blood flow since any reduction in uterine flow does not exceed a proportional reduction in oxygen requirements by the smaller uterine contents of IUGR pregnancies. Doppler velocimetry studies of the umbilical circulation have supported the interpretation of an increased resistance to flow within the umbilical vascular bed.[27, 28]

There is a striking similarity between these clinical data and those recently reported by Bell et al.[29] in heat-stressed ewes with IUGR fetuses. They found that uterine venous oxygenation decreased, with an increased venous P_{O_2} difference across the placenta. The increased venous P_{O_2} difference was a function of placental size with the smallest placentas showing the largest increase. Thus the reasons for fetal hypoxia in IUGR pregnancies are beginning to be clarified in both clincial and animal studies with a remarkable concordance in the findings.

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Long-term acid-base measurements in the fetal and maternal baboon

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OBJECTIVE: The purpose of the study was to examine the relationship between maternal and fetal acid-base indexes in the baboon.

STUDY DESIGN: Eight animals maintained in a tether system were studied during the third trimester. Results were analyzed using analysis of variance.

RESULTS: After recovery from surgery, pH in carotid artery blood of the fetus was 7.40 ± 0.027 (mean ± SD), Po_2, 27.5 ± 3.45 mm Hg; Pco_2, 34.0 ± 2.37 mm Hg; base deficit, 3.0 ± 2.15 mEq/L; Hot, 33.4% ± 2.69% and HbO_2, 61.3% ± 8.65%. The corresponding values in the mother were 7.48 ± 0.035, 100.7 ± 9.44 mm Hg, 26.4 ± 2.95 mm Hg, 3.6 ± 1.71 mEq/L, 32.6% ± 4.0% and 97.9% ± 1.28%, respectively. All indexes remained essentially the same until the onset of active labor, at which time the fetus became slightly acidotic and hypoxemic (pH < 7.35, HbO_2 < 50%).

CONCLUSION: The baboon fetus has slightly higher pH_2 and lower $Paco_2$ than the human fetus and Pao_2, base deficit, and maternal-fetal gradients across the placenta in these two primate species are comparable. (Am J Obstet Gynecol 1992;166:707-12.)

Key words: Acid-base, fetus, baboon, third trimester

Ethical considerations in humans and technical difficulties in nonhuman primates have limited the acquisition of data on fetuses in these species. Observations on the normal intrauterine environment, interrelations between mother and fetus, and responses to changes in this environment have been made most extensively in the chronically instrumented pregnant sheep. Significant progress has been made in the last decade in establishing similar methods for studies of nonhuman primates. Nonetheless, long-term data on chronically instrumented unanesthetized fetuses in primate species are still scanty.[1-3] In recent years, there have been several reports of blood sampling from the human fetus during the second and third trimesters of pregnancy.[4-6]

We now report the results of serial long-term measurements of acid-base indices in the chronically catheterized baboon fetus and its mother maintained in a tether system. The purpose of the current research is to examine the relationship between maternal and fetal arterial pH, Pco_2, Po_2, and base deficit in this species and to compare these values with those reported for the human and sheep during the third trimester of pregnancy.

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Material and methods

Data were obtained on eight pregnant baboons (*Papio papio* and *Papio anubis*) and their fetuses starting on the day of surgery and continuing until delivery. A detailed description of the maintenance of the colony, breeding, preconditioning to the tether and backpack system, anesthesia, surgery, and postoperative care were reported previously.[3]

Surgery and care. All animals were maintained in an American Association for Accreditation of Laboratory Animal Care approved facility in accordance with published guidelines[7] and met the standards of care of the Department of Agriculture. Before implementation, research protocols were approved by the Committee on Animal Care and Use at the Columbia College of Physicians and Surgeons.

The animal was first tranquilized with an intramuscular injection of ketamine (5 to 10 mg/kg) and atropine sulfate (0.2 mg). Surgery was performed under anesthesia achieved with halothane (0.025% to 2.0% as required) with a mixture of oxygen and nitrous oxide (4:6) at 132 ± 2.7 days' gestation (range 130 to 136 days, term approximately 175 days). Maternal femoral artery and vein were cannulated with Tygon microtubing (inner diameter 1.3 mm, outer diameter 2.3 mm), and the tips of the catheters were placed at the level of the aortic bifurcation and inferior vena cava, respectively. Fetal carotid artery and internal jugular

Table I. Acid-base indices (mean ± SD) in the femoral artery of eight pregnant baboons from the day of surgery (day 0) until the day before delivery (del-1).

Postop (days)	*0*	*1*	*3*	*7*	*14*	*20-22*	*Del-1*
Gestational age (days)	132.3 ± 2.71	133.4 ± 2.56	135.4 ± 3.11	139.1 ± 2.96	145.5 ± 3.63	153.6 ± 5.68	159.2 ± 10.66
pH	7.42 ± 0.042*	7.48 ± 0.019	7.48 ± 0.035	7.48 ± 0.027	7.48 ± 0.031	7.48 ± 0.041	7.49 ± 0.027
Pa_{O_2} (mm Hg)	109.5 ± 12.50*	97.5 ± 11.43	100.7 ± 9.44	98.9 ± 7.25	100.4 ± 10.9	102.8 ± 12 47	99.4 ± 8.84
Pa_{CO_2} (mm Hg)	30.3 ± 4.85	25.7 ± 4.01	26.4 ± 2.95	27.9 ± 3.35	26.6 ± 4.29	27.39 ± 3.50	27.6 ± 2.39
Base deficit (mEq/L)	4.2 ± 2.59	3.5 ± 2.80	3.6 ± 1.71	2.2 ± 1.95	2.6 ± 1.44	2.4 ± 2.23	1.8 ± 1.96*
Hematocrit (%)	35.8 ± 3.51	33.5 ± 6.11	32.6 ± 4.00	31.9 ± 5.36	30.9 ± 2.33	32.2 ± 3.81	30.7 ± 2.15
Oxygenated hemoglobin (%)	98.1 ± 1.05	97.7 ± 0.90	97.9 ± 1.28	97.3 ± 1.91	97.9 ± 1.66	98.4 ± 0.56	98.3 ± 0.84

*$p > 0.05$ Compared with day 3.

vein were cannulated with Tygon tubing (inner diameter 1.0 mm, outer diameter 1.8 mm), advanced to the level of the aortic arch and superior vena cava, respectively (4 to 6 cm). A similar catheter, but with multiple holes at the end, was attached to the fetal neck resting in the amniotic cavity to record intraamniotic pressure and replenish amniotic fluid if necessary.

All fetal catheters were brought out through the maternal peritoneum and fascia; both maternal and fetal catheters were then tunneled first to the flank of the mother and then to an infrascapular site over which the back pack was later attached.

On return of the baboon to the colony, the color-coded catheters were attached to appropriate transducers and extension tubing. This tubing was protected inside a flexible stainless steel cable that connected the backpack to a swivel device at the top of the cage. Thus continuous monitoring of pressure and sampling of blood was allowed with minimal restraint of the mother. With prior conditioning, the behavior patterns of mothers in the tether were virtually identical to those seen normally in individually caged animals.

All vascular catheters were infused continuously with normal saline solution containing 1.5 U/ml heparin and 500 U/ml aqueous penicillin at rates of 0.2 to 0.5 ml/hr to the fetus and 1 to 4.0 ml/hr to the mother, with flow pumps attached to the swivel (Holter #904 & 907, respectively). The mother was maintained on intravenous hydration through the first 24 to 72 hours, until she resumed her normal activities. Analgesia was provided with intravenous infusion of morphine sulfate at 0.1 to 1 mg/hr for at least the first three nights after surgery.

Recording and sampling. Continuous recordings were begun immediately after the animal returned from surgery. All biophysical recordings were made with appropriate preprocessors on an eight-channel strip chart recorder for on-line management and eight-channel analog tape recorder. All recording equipment was located in a room adjacent to the study cage facility.

Simultaneous maternal and fetal arterial samples of approximately 0.7 ml volume were obtained anaerobically three to five times a week at about 3:00 PM to control for the effects of any prior nocturnal uterine contractions or diurnal variation. Each sample was collected after withdrawing a volume equal to 2 to 2.5 times the volume of dead space of the catheters (1.1 ml for fetal and 1.5 for maternal) to avoid dilution or introduction of air into the samples. Samples were analyzed for hematocrit, acid-base, and blood gases (Radiometer pH Gas Monitor #ABL3C) and oxygen saturation (IL Co-oximeter #282). Coefficient of variations for repeated measures on the individual samples are 0.006 for pH, 1% for P_{CO_2}, 2% for P_{O_2}, and 3% for hemoglobin oxygen saturation.

Results are presented as mean ± SD; differences in mean values are considered significant when $p < 0.05$. Values in the mother and fetus over the third trimester were analyzed with one-way analysis of variance for repeated measures. Comparison between maternal and fetal values were made with the Student *t* test for paired samples. Correlation analyses between maternal and fetal values were peformed with the Systat computer program to determine a best fit linear regression.

Results

Maternal and fetal outcome. Postoperative uterine contractions were present in the evening hours in six of the eight animals. These usually continued for 3 to 5 days after surgery and completely disappeared thereafter in four of the above animals. In one animal, mild to moderate uterine contractions continued to appear each night until delivery at 171 days' gestation. In another animal some contractions were also present dur-

Table II. Acid-base indices (mean ± SD) in the carotid artery of eight fetal baboons from the day of surgery (day 0) until the day before delivery (del-1).

Postop (days)	*0*	*1*	*3*	*7*	*14*	*20-22*	*Del-1*
Gestational age (days)	132.2 ± 2.71	133.4 ± 2.56	135.4 ± 3.11	139.1 ± 2.96	145.5 ± 3.63	153.6 ± 5.68	159.2 ± 10.66
pH	7.32 ± 0.052*	7.38 ± 0.029	7.40 ± 0.030	7.38 ± 0.027	7.39 ± 0.026	7.39 ± 0.035	7.39 ± 0.032
Pa_{O_2} (mm Hg)	30.3 ± 3.69	25.0 ± 5.79	27.5 ± 3.45	27.3 ± 4.15	24.6 ± 4.07	25.8 ± 4.07	24.0 ± 5.57
Pa_{CO_2} (mm Hg)	40.4 ± 6.64*	32.6 ± 2.08	34.0 ± 2.37	34.5 ± 4.05	33.1 ± 4.25	34.4 ± 3.47	34.6 ± 2.08
Base deficit (mEq/L)	4.9 ± 3.04	5.0 ± 1.75	3.0 ± 2.15	3.7 ± 1.52	4.4 ± 2.38	3.4 ± 3.07	3.2 ± 2.26
Hematocrit (%)	39.5 ± 3.59†	34.7 ± 4.47	33.4 ± 2.69	33.4 ± 4.42	34.5 ± 3.39	33.4 ± 3.99	35.2 ± 3.75
Oxygenated hemoglobin (%)	57.4 ± 14.42	58.7 ± 10.16	61.3 ± 8.65	60.9 ± 8.47	56.1 ± 7.75	57.8 ± 9.31	55.7 ± 10.35

*$p < 0.05$ compared with day 3.
†$p < 0.01$ compared with day 3.

ing the daytime for the first week after surgery. Morphine was given to all animals during the first three postoperative nights; morphine was continued for four or five nights in two animals and during the daytime also in one other because of persistent uterine contractions. All fetuses were alive until time of delivery, which occurred at 160 ± 3.1 days' gestation (range 151 to 171 days); one animal was delivered by cesarean section at 169 days and the remaining seven delivered vaginally. The mean duration of these studies was 28 ± 6.9 (range 21-39 days).

Maternal acid-base status. Mean acid-base indices in arterial blood of the eight pregnant baboons from the day of surgery to the day preceding delivery are given in Table I. Immediately after surgery the pregnant baboon showed evidence of mild metabolic acidosis, which in most cases was absent by the fourth or fifth postoperative day when the animal had resumed a normal diet and overnight uterine contractions had subsided. Values during the subsequent days reflected a state of slight respiratory alkalosis. Apart from a tendency for hematocrit to decrease with gestation, all values remained essentially unchanged throughout the period of study (r values ranging from 0.13 to 0.41 and p values >0.1).

Fetal acid-base status. Fetal arterial pH, blood gas, and hematocrit values are summarized in Table II. On the day of surgery fetal pH was significantly lower than subsequent values while Pa_{CO_2} and hematocrit were higher. Base deficit remained high for the first 2 to 3 days. Subsequent fetal arterial pH, Pc_{O_2}, base deficit, and hematocrit values did not change significantly during the third trimester (r values for correlation with gestation ranged from 0.07 for Pc_{O_2} to 0.41 for Po_2 and p values >0.1); the ranges were as follows: pH 7.33 to 7.43, Pa_{CO_2} 29 to 39 mm Hg, base deficit 7 to −0.6 mEq/L, and hematocrit 31% to 39%. Fetal Pa_{O_2} was higher on the day of surgery than during the following days, when it stabilized; however, fetal oxygenation was essentially unchanged throughout the period studied.

Fetal-maternal gradient for pH remained remarkably stable, being −0.095 ± 0.037 on the first postoperative day and −0.096 ± 0.015 on the day before delivery. Gradients for Pa_{CO_2} were 6.9 ± 4.29 on day 1 and 7.0 ± 3.19 mm Hg the day before delivery; the corresponding gradients for base deficit were 1.5 ± 2.08 and 1.4 ± 1.70 mEq/L, respectively. When values obtained on the day of surgery and during active labor were excluded, there was a strong, positive correlation between maternal and fetal pH ($r = 0.693$, $p = 0.001$), Pc_{O_2} ($r = 0.757$, $p < 0.001$), base deficit ($r = 0.680$, $p = 0.008$), and Po_2 ($r = 0.635$, $p = 0.021$ but only when animal #433 depicted in Fig. 2 was excluded; see below).

Serial acid-base values in arterial blood of baboon #406 and her fetus are shown in Fig. 1. This animal had little or no uterine activity after surgery (at 131 days) until two nights before delivery (at 152 days). Immediately after surgery the mother had a relative respiratory acidosis compared with the normal value during pregnancy (pH = 7.406, Pa_{CO_2} 40.7); however, the values returned to normal by the first postoperative day and remained unchanged until the onset of active labor. The range of pH was 7.47 to 7.51, Pa_{O_2} 90-110 mm Hg, Pa_{CO_2} 26 to 34 mm Hg, and base deficit 0.5 to 4 mEq/L. During active labor and delivery the animal developed a moderate metabolic acidosis (base deficit 6.2 mEq/L). The fetus had both respiratory and metabolic acidosis immediately after surgery: pH 7.25, Pa_{CO_2} 47 mm Hg and base deficit 7 mEq/L. Values returned to normal within 2 days and remained stable until labor was established. The average normal values

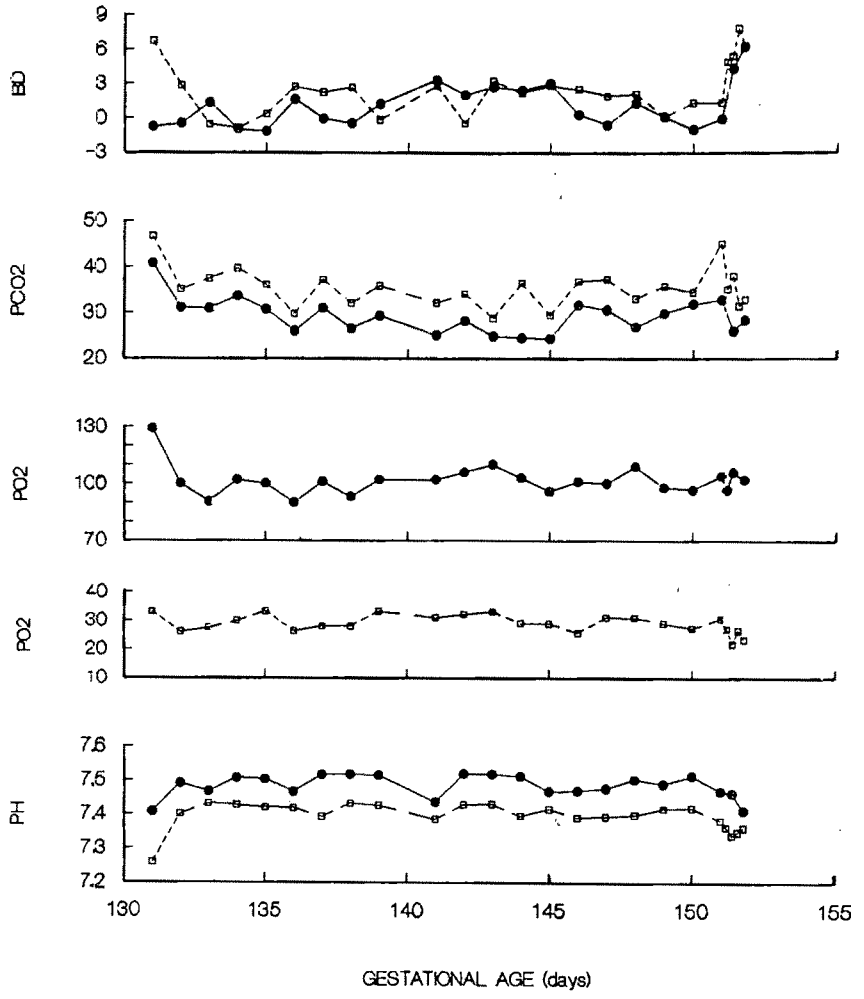


Fig. 1. pH, Pa_{O_2}, and base deficit in baboon #406 and her fetus from day of surgery at 131 days until delivery on day 152. This animal exhibited very mild or no uterine contractions until day 150. □--□, Fetus; ●——●, mother.

were pH 7.42 ± 0.022, Pa_{O_2} 30 ± 2.5 mm Hg, Pa_{CO_2} 34 ± 3.2 mm Hg, and base deficit 1.5 ± 1.52 mEq/L. Fetal acidosis and hypoxemia developed with the establishment of labor. The lowest values for pH (7.34) and Pa_{O_2} (22 mm Hg) and highest values for Pa_{CO_2} (45 mm Hg) and base deficit (8 mEq/L) occurred about 1 hour before delivery. Maternal-fetal gradients in acid-base indices were greatest on day of surgery and at delivery, whereas during the remainder of the study period most of the daily variations in fetal acid-base could be accounted for by those of the mother.

Fig. 2 shows daily acid-base values in baboon 433. This animal had mild to moderate uterine contractions (pressures of 25 to 40 mm Hg at a rate of 3 to 4/10 minutes) every night (for 3 to 5 hours) until delivery at day 171. Maternal Pa_{O_2} values in this animal were in the same range as in animal 406; however, pH and Pa_{CO_2} were slightly lower (range 7.44 to 7.49 for pH and 24 to 31 mm Hg for Pa_{CO_2}), whereas base deficit was slightly higher (2 to 6 mEq/L). Fetal pH, Pa_{CO_2}, and base deficit stabilized by postoperative day 2; however, as in the mother, pH and Pa_{CO_2} were slightly lower (7.38 ± 0.020 and 31 ± 2.3), whereas base deficit was higher (6 ± 1.4 mEq/L). On the other hand, Pa_{O_2} showed a gradual decline from 27 to 29 mm Hg a few days after surgery to values as low as 18 to 21 mm Hg in the days before delivery. In general, maternal-fetal gradients of the various indices for this animal were greater than those in other animals who had minimal uterine contractions during the period of the observations.

Comment

The present long-term observations were obtained from chronically catheterized fetal baboons and their mothers maintained under stable conditions with a tethering and backpack system described previously.[5] During the third trimester, acid-base indices in fetal

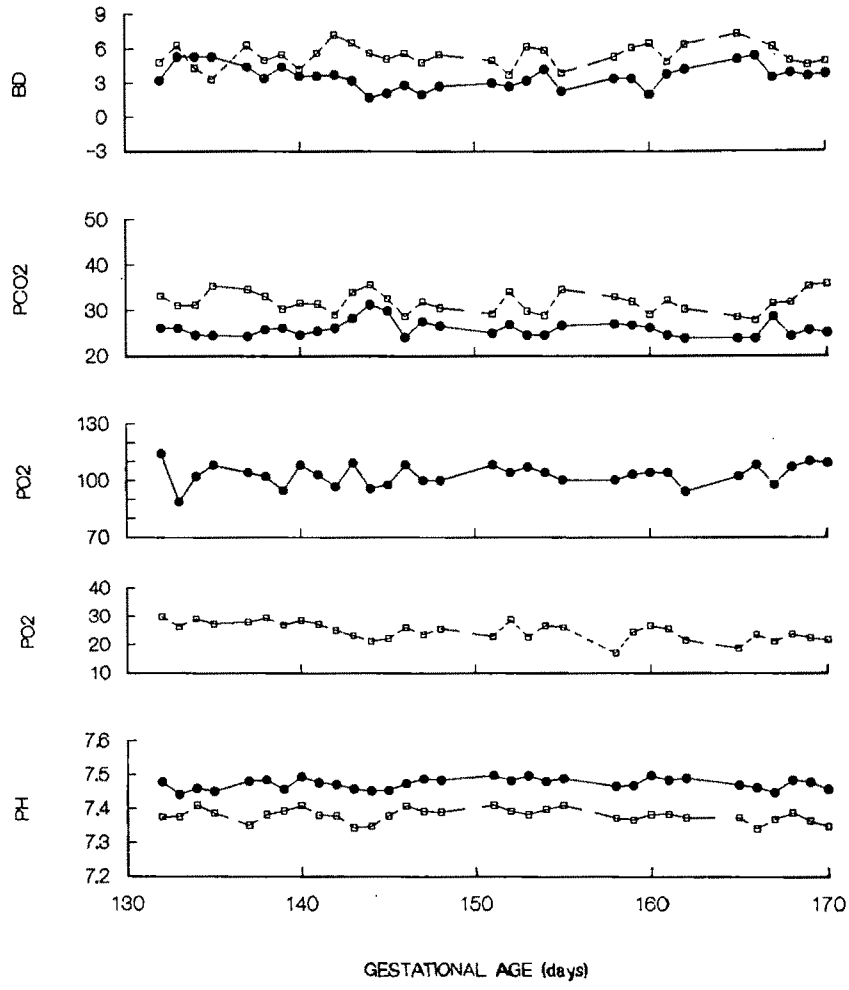


Fig. 2. pH, Pa_{O_2}, Pa_{CO_2}, and base deficit in baboon 433 and her fetus from day of surgery at 132 days until day before delivery at 171 days. This animal had uterine contractions (30-40 mm Hg height and 3 to 4/10 mins) for ≥4 hours every evening. □--□, Fetus; ●——●, mother.

carotid artery, with the exception of P_{O_2}, were within the normal range for adult animals. These indices, including P_{O_2}, stabilized within 1 to 2 days after surgery and remained essentially unchanged until the onset of active labor. As expected, data demonstrate a consistently lower arterial pH in the fetus (average −0.095), a higher P_{CO_2} (average 7 mm Hg), and a higher base deficit (average 1.5 mEq/L) than the corresponding values in the mother.

When compared with values reported in pregnant sheep, our results are similar with regard to pH; however, P_{CO_2} values are slightly lower and base deficit and P_{O_2} higher in the pregnant baboon.[8-12] It is possible that these differences are related to the overnight uterine contractions that occurred in some animals. In addition, although the baboons were extensively preconditioned to the tethering system and appeared undisturbed during the sampling of blood, the presence of a small, combined respiratory alkalosis and metabolic acidosis could be due to some degree of agitation. However, levels of catecholamines, vasopressin and β-endorphin in maternal plasma were within the range of unstressed, pregnant animals.[3, 12] Fetal P_{O_2} values in the carotid artery were 8 to 10 mm Hg higher in the baboon than the corresponding values in the sheep fetus; however, because of the differences in dissociation curves, this was not reflected in hemoglobin oxygen saturation. The baboon fetus also had higher pH and base deficit and lower Pa_{CO_2} values when compared with those found in the fetal sheep. Maternal-fetal gradients of pH, Pa_{CO_2}, and P_{O_2} but not base deficit were generally smaller in the baboon than those reported for the sheep. This may reflect a higher diffusion rate for carbon dioxide and oxygen across the placenta in the baboon.

The range of pH values in humans taken from the umbilical artery of the fetus at the time of cordocentesis during the second and third trimesters is reported to

be 7.32 to 7.37, whereas the range in maternal arterial or free flowing venous blood taken at the same time is 7.36 to 7.45.[4-6] These values are lower than those we obtained in the baboon. The ranges for P_{CO_2} reported in humans are generally higher than the present results on the baboon, whereas values for P_{C_2} and base deficit in the two primate species appear to be in the same range for both the mother and the fetus. Whether these differences are because sampling in the human necessitates some degree of manipulation or because the baboons may be slightly agitated cannot be determined at the present time. It should also be pointed out that fetal samples in the present study were taken from the carotid artery during the third trimester; the results reported on human fetuses were taken from the umbilical artery during midgestation. P_{O_2} values in the umbilical artery are expected to be 2 to 4 mm Hg lower than those in the carotid artery, and there is a tendency for fetal P_{O_2} values to fall with gestational age.[5, 6, 13] In spite of these differences, maternal-fetal gradients for all indices are essentially the same in both species, reflecting the similarities in placental transport of components of acid-base.

Apart from preliminary results we published earlier, there are no reported data on acid-base value in the chronically instrumented, unanesthetized pregnant baboon and its fetus.[3] The only values in the chronically instrumented nonhuman primate fetus were reported by Jackson et al.[2] in the rhesus monkey. These are very similar to human data; however, the animals were sedated with ketamine or sernylan for the purpose of the study.

Reports in humans and more recently in sheep have shown that there is usually a decrease in fetal P_{O_2} and an increase in hemoglobin from the second to the third trimester without a change in oxygen content.[5, 6, 13] We found no such trend in our animals. However, our observations were all obtained during the third trimester (average duration 21 to 39 days). There was a tendency for Pa_{O_2} to fall in the one animal (Fig. 2) observed for the longest period of time; this animal had mild uterine contractions during the evening throughout the study. It is therefore not possible to determine whether this decrease in Pa_{O_2} and lack of correlation with maternal Pa_{O_2} were due to the periodic reduction in uterine blood flow or represented a true tendency for diminished arterial oxygen tension during the third trimester in the baboon fetus.

In conclusion, our results demonstrate that acid-base indices of the unanesthetized fetal and maternal baboon maintained in a tether system stabilize by the third postoperative day and remain unchanged throughout the third trimester until the onset of active labor. Although Pa_{O_2} and base deficit in both mother and fetus are comparable with those in humans, fetal pH is higher and Pa_{CO_2} is lower. Maternal-fetal gradients of these values, however, are the same in the two species. It is speculated that these differences are due to the conditions under which samples were obtained rather than true species differences.

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The effects of glycemia on breathing movements and plasma prostaglandin E concentrations in the sheep fetus

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OBJECTIVE: The purpose of this study was to determine the roles of fetal plasma glucose and prostaglandin E in controlling fetal breathing movements.

STUDY DESIGN: Five late pregnant ewes with catheterized twin fetuses were fasted for 48 hours; one fetus was kept normoglycemic (by glucose infusion) and the other allowed to become hypoglycemic during this period. Fetal breathing movement and fetal and maternal plasma prostaglancin E and glucose concentrations were measured throughout. Data were analyzed by paired and unpaired *t* tests.

RESULTS: The mean incidence of fetal breathing movement was significantly greater in the normoglycemic fetuses that in the hypoglycemic twins at the end of the fast ($p < 0.05$), whereas plasma prostaglandin E levels increased significantly ($p < 0.05$) and to the same extent in both groups of fetuses.

CONCLUSION: The reduced incidence of ovine fetal breathing movement that occurs during maternal fasting appears to be primarily caused by fetal hypoglycemia and does not directly involve changes in fetal plasma prostaglandin E. (AM J OBSTET GYNECOL 1992;166:713-9.)

Key words: Fetal breathing, hypoglycemia, prostaglandin E

Breathing movements are a normal feature of fetal life. They occur episodically throughout the latter half of gestation and are related to the metabolic and endocrine status of the fetus.[1] In particular, the circulating glucose concentration appears to be an important influence on fetal breathing activity in late gestation.[1, 2] In the fetus sheep near term, hyperglycemia increases the incidence of fetal breathing movements, whereas hypoglycemia, induced by fasting or insulin infusion, decreases fetal respiratory activity.[3-6] Similar observations have been made in the human infant during late gestation.[2]

Although fetal breathing activity appears to be closely related to glucose availability in utero, it is still not clear whether these changes in fetal breathing movements are due to variations in the fetal glucose concentration per se or whether other nutritional or hormonal changes are involved. Certainly, infusions of glucose into hypoglycemic fetuses of fasted ewes restores the normal incidence of fetal breathing movements, but the glucose levels achieved in these experiments were twice the normal fetal concentrations.[5] More recent studies have suggested that the low incidence of fetal breathing movements seen during fetal hypoglycemia may be due, in part to a concomitant increase in the prostaglandin E_2 (PGE_2) concentration in fetal plasma.[6]

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Prostaglandins have major effects on fetal breathing activity in the sheep.[7] Infusion of prostaglandins, particularly PGE_2, reduces the incidence of fetal breathing movements in sheep, whereas conversely, inhibition of prostaglandin synthesis stimulates fetal breathing activity.[8-10] Prostaglandin production in utero is also known to be related to maternal nutritional state.[11, 12] Increases in uterine prostaglandin synthesis occur during dietary restriction, and an inverse relationship between the plasma prostaglandin and glucose concentrations is observed in the fetus and pregnant ewe when nutrient availability is manipulated during late gestation.[12, 13] Hence in this study the roles of plasma prostaglandin E (PGE) and glucose in controlling fetal breathing movements have been examined more closely by comparing fetal breathing movements and plasma PGE concentrations during maternal fasting in twin sheep fetuses in which one fetus was allowed to become hypoglycemic while the other was maintained in a normoglycemic state by fetal glucose infusion.

Material and methods

Animals. Five Welsh Mountain ewes carrying twin fetuses of known gestational age were used. All 10 lambs were alive at delivery, which occurred at a mean gestational age of 143.2 ± 1.2 days ($n = 5$). Mean birth weight was 2.135 ± 0.063 kg ($n = 10$).

Operative procedures. Between 112 and 117 days of gestation, anesthesia was induced with thiopental and maintained with halothane (1.5% in nitrous oxide and oxygen) after intubation. With established procedures,[12, 14] catheters were inserted into the carotid artery, jugular vein, trachea, and amniotic sac of each

twin fetus and into a uterine vein and dorsal aorta of the ewe. Antibiotics were given intravenously to the fetus (100 mg ampicillin [Penbritin]) and intramuscularly to the mother (10 ml Streptopen) at the end of surgery. Normal feeding patterns were generally restored within 24 to 48 hours of operation, but no experiments were carried until 12 to 21 days after surgery.

Experimental procedures. Food but not water was withdrawn from the ewes for 48 hours beginning at 131 to 136 days of gestation at either 9 AM ($n = 2$) or 5 PM ($n = 3$). Glucose was infused intravenously into one twin of each pair at 300 mg/hr for the first 24 hours, 600 mg/hr for the next 12 hours, and then at 900 mg/hr for the last 12 hours of the 48-hour fast. The twin to be infused was chosen randomly.

During the experimental period, 8 ml blood samples were taken from the uterine vein and fetal and maternal arteries either between 9 and 10 AM ($n = 2$) or 4 and 5 PM ($n = 3$) starting 24 hours before the fast was begun and continuing until 24 hours after the animals were refed. A small aliquot of blood (0.5 ml) was used for analyses of pH, P_{O_2}, P_{CO_2}, hemoglobin concentration, and oxygen saturation with Radiometer equipment (ABL 330 and OSM2, Radiometer American, Inc., Westlake, Ohio). Blood oxygen content was calculated from the hemoglobin and oxygen saturation values. Two milliliters of blood was added to chilled tubes containing aspirin (1.4 mg) and ethylenediamine tetraacetic acid (0.10 mg) for analysis of PGE. These samples were centrifuged at 4° C, and an equal volume of 0.12 mmol/L methoxyamine hydrochloride in sodium acetate buffer (1 mol/L, pH 5.6) was added to the plasma. Plasma samples with added methoxyamine hydrochloride were left at room temperature for 24 hours then frozen at −20° C until analysis. The remainder of the blood samples (≈5 ml) were added to tubes containing ethylenediamine tetraacetic acid and centrifuged at 4° C. The plasma from these samples was stored at −20° C until required for metabolite and other hormonal analyses.

Recordings of fetal breathing movements were made for 4 to 6.5 hours between 10 AM and 4:30 PM on the day before, the day after, and the 2 days of the 48-hour fast. Tracheal and amniotic fluid pressures were measured in each fetus with standard pressure manometers and displayed on an eight-channel Lectromed recorder together with the electronically derived difference between the tracheal and amniotic pressures. All operative and experimental procedures were approved and licensed by the appropriate authorities.

Biochemical analyses. Glucose concentrations were measured in plasma that was deproteinized with zinc sulfate (0.3 mol/L) and barium hydroxide (0.3 mol/L) with glucose oxidase.[12] Plasma PGE was assayed by radioimmunoassay against PGE_2 methyloxime standards with use of an antiserum raised in goats against the methyloxime of PGE_2.[15] Detailed procedures and specificity of the PGE assay for ovine plasma have already been published.[12] The limit of sensitivity of the assay was 0.5 nmol/L, and intraassay and interassay coefficients of variation were ≤10% and 21.4%, respectively, for the assays required for this study. Because the antiserum did not distinguish between PGE_1 and PGE_2,[12] plasma PGE concentrations have been expressed as PGE_2 equivalents.

Data analyses. An episode of fetal breathing movements was defined as a series of negative deflections in tracheal pressure, from which amniotic pressure had been subtracted, that were >0.8 mm Hg and lasted longer than 0.5 minute. The incidence of fetal breathing movements was calculated as the total amount of time within a recording period (4 to 6.5 hours) occupied by fetal breathing movements and expressed as a percentage. The amplitude of the negative pressure deflections (tracheal pressure − amniotic pressure) was measured every 20 or 30 seconds during each episode of fetal breathing movements with a Cal Comp digitizing tablet with the Sigma scan program (Jandel Scientific, Corte Madera, Calif.), and the mean value was calculated for all episodes of fetal breathing movements during a recording period in each fetus.

Mean ± SEM are used throughout and statistical analyses were made according to the methods of Armitage.[16] Statistical significance was assessed by paired and unpaired t tests. Linear regression was also used to assess the relationship between fetal breathing movements and the plasma concentrations of glucose and PGE. In one glucose-infused fetus the infusion pump became detached for 8 hours during the last 12 hours of the 48-hour period of food withdrawal. Consequently, data obtained from this fetus at 48 hours of fasting have not been included in the mean values for the glucose-infused fetuses at the end of the fast.

Results

Basal values. Before the fast, fetal blood gas tensions, pH, and oxygen content were within the normal range of values,[14] as were the plasma concentrations of glucose and PGE in the fetus (Table I) and ewes (2.98 ± 0.26 mmol/L and 11.60 ± 1.11 nmol/L, respectively, $n = 5$). The incidence and amplitude of fetal breathing movements before the fast were also similar to values reported previously for fetal sheep in late gestation (Table I).[1] Before the fast began, there were no significant differences in plasma glucose, PGE, blood gas status, or incidence or amplitude of fetal breathing movements between the pairs of fetuses that were subsequently glucose infused or uninfused during the fast (Table I).

Effects of nutritional manipulation

Metabolite and hormone concentrations. Fasting significantly reduced plasma glucose levels in the mother and

Table I. Values (mean ± SE) for arterial blood gas tensions, pH, oxygen content, plasma concentrations of glucose and PGE, and mean incidence and amplitude of fetal breathing movements before fasting in twin sheep fetuses that were subsequently uninfused ($n = 5$) or infused with glucose ($n = 5$) during a 48-hour period of maternal fasting

	Arterial plasma concentration		*Arterial*				*Fetal breathing movements*	
Animals	*Glucose (mmol/L)*	*PGE (nmol/L)*	*pH*	*Po_2 (mm Hg)*	*Pco_2 (mm Hg)*	*Oxygen content (mmol/L)*	*Incidence (%)*	*Amplitude (mm Hg)*
Uninfused	0.74 ± 0.10	4.62 ± 1.31	7.367 ± 0.009	19.0 ± 0.7	48.2 ± 1.3	3.50 ± 0.28	47.7 ± 5.7	3.9 ± 0.4
Glucose-infused	0.79 ± 0.09	3.57 ± 1.32	7.364 ± 0.006	21.7 ± 1.2	46.6 ± 1.2	3.79 ± 0.24	44.4 ± 5.4	4.7 ± 0.9

Table II. Values (mean ± SE) for arterial plasma concentrations of glucose and PGE before, during, and after 48-hour period of food withdrawal begun at time 0 in ewes and their twin fetuses, which were either glucose infused or uninfused during fast

		Time from food withdrawal				
	No. of animals	*−24 hr*	*0 hr*	*24 hr*	*48 hr*	*72 hr*
Glucose (mmol/L)						
Uninfused fetuses	5	0.70 ± 0.12	0.77 ± 0.10	0.51* ± 0.06	0.48*† ± 0.06	0.65 ± 0.08
Infused fetuses	4	0.83 ± 0.12	0.84 ± 0.08	0.68 ± 0.07	0.81 ± 0.06	0.66 ± 0.08
Mothers	5	3.05 ± 0.28	2.92 ± 0.34	1.89* ± 0.18	1.76* ± 0.18	2.72 ± 0.48
PGE (nmol/L)						
Uninfused fetuses	4	5.14 ± 2.44	6.44 ± 2.24	6.91 ± 1.51	8.30* ± 2.54	6.24 ± 1.51
Infused fetuses	4	4.15 ± 2.04	3.30 ± 1.65	5.46 ± 1.87	6.60* ± 1.92	4.49 ± 1.76
Mothers	4	10.10 ± 0.85	11.13 ± 1.33	13.51 ± 0.27	18.19 ± 2.57	16.55 ± 2.70

*Significantly different from time 0 value, $p < 0.05$ (paired t test).
†Significantly less than value in infused fetuses, $p < 0.01$ (paired and unpaired t test).

uninfused fetus at 24 and 48 hours (Table II). Infusion of glucose into the other fetus of the pair maintained normoglycemia; plasma glucose levels were not significantly different from prefasting values at 24 and 48 hours and were significantly greater than those in the uninfused fetuses after 48 hours of fasting (Table II). In the fetus in which glucose infusion failed, the plasma glucose level was maintained for the first 24 hours of maternal fasting but had fallen to a value similar to that in the uninfused fetuses by the end of the 48-hour fast. After refeeding was carried out for 24 hours, plasma glucose levels in the mothers and uninfused fetuses were not significantly different from prefasting values (Table II).

Maternal fasting led to increases in plasma PGE concentrations in the ewes and uninfused, hypoglycemic fetuses, as reported previously.[12] The increment in maternal arterial plasma PGE during fasting varied widely between individuals (range 2.90 to 15.16 nmol/L), and hence the mean change in plasma PGE was not significant, even at the end of the 48-hour period of fasting ($p > 0.05$, Table II). Significant increases in fetal plasma PGE levels were observed at the end of the fast in both the uninfused and glucose-infused fetuses (Table II). The increment in fetal plasma PGE from the mean prefasting value was similar in the uninfused (+2.52 ± 0.53 nmol/L, $n = 4$) and glucose-infused (+2.80 ± 0.74 nmol/L, $n = 4$) fetuses. There were also no significant differences in the absolute PGE concentrations between the two groups of fetuses at any time during the experimental period (Table II). Refeeding the ewes lowered plasma PGE concentrations in the mother and both groups of fetuses; the values observed 24 hours after refeeding were not significantly different from those measured before fasting began (Table II).

No significant changes in fetal blood gas tensions, pH, or oxygen content occurred during maternal fasting in either the glucose-infused or uninfused groups of fetuses ($p > 0.05$ all cases).

Fetal breathing movements. In common with previous studies[3, 12] the incidence of fetal breathing movements decreased significantly during maternal fasting in the fetuses that became hypoglycemic (Fig. 1). The mean reduction in the incidence of fetal breathing movements during the 48-hour period of fasting was 58% ± 11.0% ($p < 0.01$) in these five fetuses. By contrast, there was no significant change in the incidence of fetal breathing movements during maternal fasting in the glucose infused fetuses (Fig. 1); the mean inci-

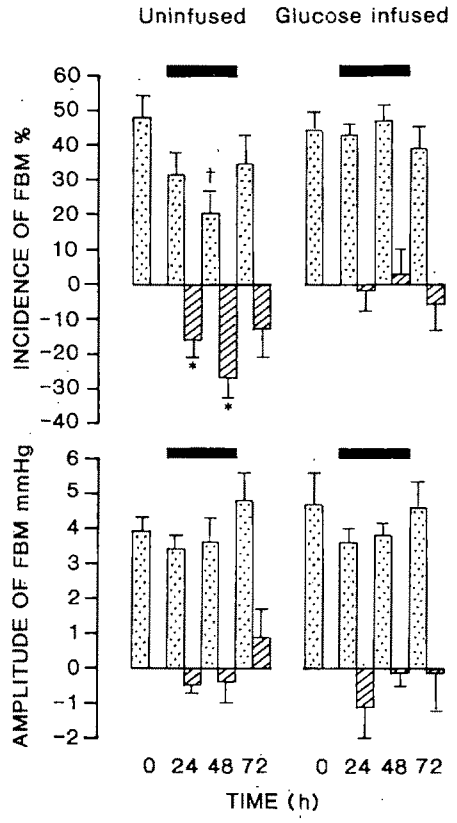


Fig. 1. Incidence and amplitude (mean ± SE) of fetal breathing movements (FBM) *(dotted bars)* before, during, and after 48-hour period of maternal food withdrawal *(solid horizontal bars)* and mean changes in value *(hatched bars)* from time 0 in fetuses uninfused ($n = 5$) or infused with glucose during fast ($n = 5$, except at 48 hours when $n = 4$). *Asterisk*, Significant change from time 0 value, $p < 0.05$; *Dagger*, Significantly different from infused fetuses, $p < 0.05$.

dence of fetal breathing movements in these fetuses was therefore significantly greater than that in the uninfused fetuses at the end of the fast (Fig. 1). After refeeding for 24 hours, the incidence of fetal breathing movements was not significantly different from prefasting values in either uninfused or glucose-infused groups of fetuses (Fig. 1). In the fetus in which glucose infusion failed, the incidence of fetal breathing movements was maintained at a normal value (38.2%) at 24 hours of fasting but had fallen to a value (13.7%) similar to that in the uninfused, hypoglycemic fetuses at the end of the fast (Fig. 1).

In contrast to earlier findings,[12] there was no apparent change in the amplitude of fetal breathing movements during maternal fasting in the hypoglycemic fetuses (Fig. 1). Nor was there any significant change in amplitude in the glucose-infused fetuses during the 48-hour period of maternal fasting (Fig. 1).

Relationship between fetal breathing movements and plasma concentrations of glucose and PGE. When the data from all the fetuses were combined, irrespective of the day or type of treatment, there was a significant positive correlation between the incidence of fetal breathing movements and the arterial plasma glucose concentration in the individual fetuses (Fig. 2). No significant correlation was observed between the incidence of fetal breathing movements and the fetal plasma PGE level ($r = -0.120$, $n = 32$, $p > 0.05$) or between the latter values and the fetal arterial concentration of plasma glucose ($r = -0.166$, $n = 32$, $p > 0.05$).

Comment

This study demonstrates that the reduction in the incidence of fetal breathing movements observed during maternal fasting is due primarily to the fall in glucose concentration in the fetal plasma. The degree of fetal hypoglycemia produced by fasting and the reduction in breathing activity observed in the hypoglycemic fetuses in the current study were similar to those reported previously, as were the increments in plasma PGE.[3, 4, 6, 12] When fetal normoglycemia was maintained during the fast by fetal glucose infusion, there was no reduction in the incidence of fetal breathing movements in spite of an increase in fetal plasma PGE similar to that seen in the hypoglycemic fetuses. These observations indicate that, contrary to previous suggestions,[6] PGE is not directly involved in regulating fetal breathing movements during maternal fasting, although any effects of PGE may have been obscured by glucose administration in the fetus.

Role of prostaglandins. In other circumstances prostaglandins, including PGE, are known to affect the incidence of fetal breathing movements in the sheep.[7] Reducing the circulating prostaglandin levels in the fetus by treatment with prostaglandin synthetase inhibitors (aspirin, indomethacin, meclofenamate) leads to an increased incidence of fetal breathing movements, which can be restored to its normal value by PGE infusion.[7, 10] Intrafetal infusion of prostaglandin, particularly PGE, to levels similar to those seen during labor also causes a rapid reduction in the normal incidence of fetal breathing movements.[10] In the current study the percentage increase in fetal plasma PGE observed during fasting was smaller than that during infusions of PGE sufficient to reduce the incidence of fetal breathing movements.[10] Furthermore, fetal plasma PGE concentrations observed at the end of the fast in this study were less than those measured during labor.[12] These observations suggest that there may be a critical circulating concentration of PGE above which inhibition of fetal breathing movements occurs. The finding that the relationship between the incidence of fetal breathing movements and the dose of PGE_2 infused into meclofenamate-treated fetuses is not linear supports this suggestion.[10]

Previous studies have shown that the concentrations of plasma glucose and PGE are both significant influ-

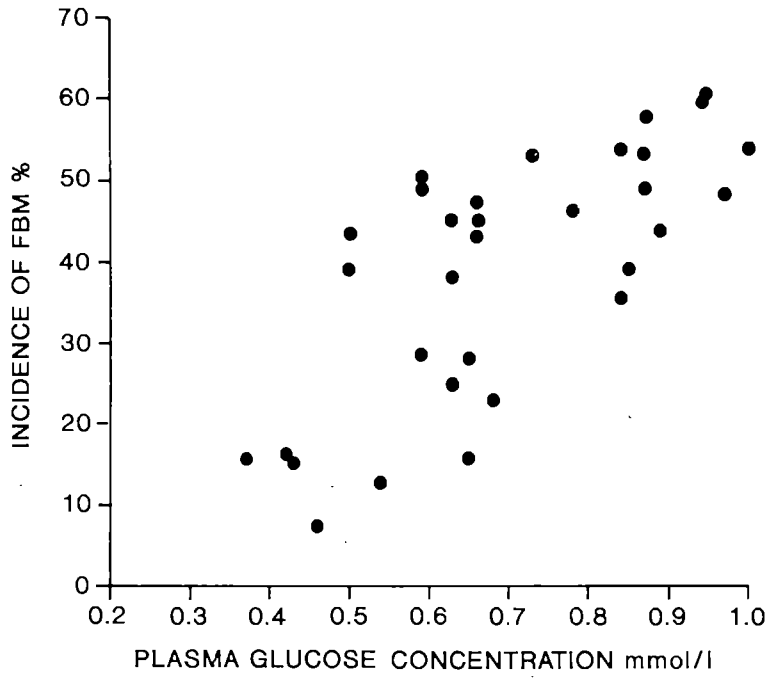


Fig. 2. Relationship between incidence of fetal breathing movement (FBM) and arterial concentration of plasma glucose in individual fetuses, irrespective of their nutritional state. $y = 62.5x - 4.6$, $n = 32$, $r = 0.716$, $p < 0.01$.

ences on the incidence of fetal breathing movements in the sheep fetus after 130 days of gestation.[10,12] Under normal nutritional conditions, PGE may be the more important factor physiologically as fetal PGE levels rise toward term, whereas fetal plasma glucose levels vary very little during late gestation in well-fed animals.[17,18] However, even under these conditions, PGE is unlikely to be the only influence on fetal breathing movements because the decrease in breathing activity observed toward term still occurs when the normal prepartum increase in fetal plasma PGE is abolished by meclofenamate treatment.[19] Certainly in the current study fasting-induced rises in plasma PGE did not inhibit fetal breathing activity when they were accompanied by fetal normoglycemia, which suggests that plasma glucose may be the more important factor regulating fetal breathing movements during adverse nutritional conditions.

Central mechanisms. The mechanism whereby hypoglycemia reduces the incidence of fetal breathing movements is still unclear but may involve changes in cerebral metabolism and acid-base balance.[2,3,7] Fetal hypoglycemia, induced by insulin administration, reduces cerebral glucose utilization and leads to changes in the acid-base status of the peripheral circulation.[5,6] On the other hand, hypoglycemia induced by fasting has no apparent effect on cerebral glucose consumption, although there is a reduction in sagittal sinus P_{CO_2} and H^+ ion levels, which suggests that less carbon dioxide is being produced by the fetal brain under these circumstances.[3] Certainly, when glucose is infused into hypoglycemic fetuses of fasted ewes, there is a rise in cerebral glucose utilization that is accompanied by central acidosis and an increase in fetal breathing activity.[3] These observations suggest that fetal glycemia may alter fetal breathing activity by changing the chemical environment of the central chemoreceptors. These receptors are responsive in utero and known to be active in the control of ovine fetal breathing movements during late gestation.[20,21]

It has also been postulated that central chemoreceptors mediate the respiratory effects of prostaglandins in the fetus.[7,21,22] Infusions of prostaglandin synthetase inhibitors produce central acidosis in the sheep fetus, whereas PGE infusion may cause cerebral vasodilatation and lead to central alkalosis as a consequence.[22] Changes in the fetal concentrations of PGE and glucose may therefore act on fetal breathing movements through a common pathway, which, in the case of maternal fasting, would have additive effects at the chemoreceptors and lead to a reduced incidence of fetal breathing movements. However, the effect of the increment in PGE induced by fasting must be relatively small, as no change in fetal breathing movements was observed in the current study when fetal PGE levels increased in the absence of any change in fetal glucose concentrations. Whatever the central mechanisms involved, these findings show that the respiratory effects of fetal hypoglycemia, induced by fasting, are not mediated through changes in fetal plasma PGE.

Uteroplacental prostaglandin synthesis. Hypoglycemia in either the mother or fetus appears to be a potent stimulus to prostaglandin synthesis in the pregnant ewe. When maternal glucose levels fall during fasting or insulin administration, there are increases in the maternal concentrations of PGE and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM), the main metabolite of prostaglandin $F_{2\alpha}$.[11-13] Similarly, when the fetus becomes hypoglycemic, its prostaglandin levels rise even when there is no change in maternal glycemia.[11-13] The most likely source of this PG is the uteroplacental tissues, because there are significant arteriovenous concentration differences in PG across both sides of the ovine placenta.[11, 12, 23] Furthermore, these concentration differences across the uterine circulation widen during hypoglycemia.[11-13]

The present finding that fetal plasma PGE levels increased during fasting in both twins, irrespective of their glucose concentrations, appears to suggest that maternal glucose levels may have the more dominant role in regulating uteroplacental PG production. However, when glucose is infused into single fetuses to maintain normoglycemia during maternal fasting, no rise in maternal plasma PGFM occurs in spite of sustained hypoglycemia in the ewe.[24] Although neither $PGF_{2\alpha}$ nor PGE was measured in this study,[24] these findings suggest that uteroplacental prostaglandin production can be inhibited by glucose infusion into a single fetus. However, infusion of glucose into one of a pair of fetuses, even at rates higher than its normal use, clearly is not sufficient to overcome the combined effects of hypoglycemia in its mother and uninfused twin. Increased PGE production therefore occurs in the uteroplacental tissues of the uninfused twin and leads directly to elevated PGE levels in the ewe and hypoglycemic fetus. Because PGE crosses the ovine placenta[25] and maternal PGE concentrations are invariably higher than those in the fetus, the high plasma PGE level found during fasting in the normoglycemic fetus could be due merely to transplacental passage of PGE down its concentration gradient from the ewe.

The way in which glycemia affects prostaglandin synthesis remains unknown, although the results of this and previous studies indicate that the rate of placental glucose consumption is probably a key factor in determining prostaglandin production by the uteroplacental tissues.[12, 24] However, further studies of uteroplacental prostaglandin output in different nutritional conditions are required before the role of placental glucose consumption and the relative importance of fetal and maternal glucose in controlling prostaglandin synthesis can be ascertained fully. It may be that changes in the availability of other metabolites such as fatty acids, which accompany the changes in glycemia induced by fasting, are also important in determining prostaglandin production in utero.[11]

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Human vaginal leukocytes and the effects of vaginal fluid on lymphocyte and macrophage defense functions

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Boston, Massachusetts

OBJECTIVES: The purpose of this study was to quantify, characterize, and further define the role of vaginal white blood cells in defense mechanisms and human immunodeficiency virus infection.
STUDY DESIGN: Vaginal lavages were obtained from five hea thy women throughout three menstrual cycles. Lymphocyte subpopulations, macrophages, and granulccytes were characterized and quantified by an immunohistologic technique. Vaginal lavage fluid was addec to peripheral blood mononuclear cells, and effects on cell viability, lymphocyte proliferation, macrophage phagocytosis, and expression of various cell surface molecules critical to immunologic functions were assessed. Data were analyzed by Student's *t* test.
RESULTS: Few lymphocytes were found at any stage of the menstrual cycle; however, granulocytes and macrophages were abundant at menstruation and present at low levels through the proliferative phase. Vaginal lavage fluid collected during menses, at midcycle, and after coitus suppressed mitogen-induced lymphocyte proliferation but had no effect on surface expression of human leukocyte antigen or CD4 antigens, or on macrophage function. Likewise, low pH (<5.0) medium significantly inhibited lymphocyte proliferation but had no effect on macrophage phagocytosis. The spermicide nonoxynol 9 was toxic to both lymphocytes and macrophages.
CONCLUSION: White blood cells, including lymphocytes and macrophages, are infrequently present in cervicovaginal secretions of healthy women except during menses; the vaginal environment may effect their function. (Am J Obstet Gynecol 1992;166:720-6.)

Key words: Vagina, lymphocyte, macrophage, immune defense, human immunodeficiency virus type 1

Vaginal fluid is primarily derived from cervical secretions, exfoliated epithelial cells, bacterial products, and plasma transudation through the vaginal mucosa. Uterine, oviductal, follicular, and peritoneal fluid may also contribute.[1] Sexually communicable pathogens are transmitted through this vaginal milieu. A first line of defense is the mucosal secretory immune system of the cervix, which is capable of producing abundant amounts of secretory immunoglobulin A.[2] Cellular mechanisms important in host defense against pathogenic bacteria and viruses in the vagina include cytotoxic T-cell, natural killer cell, macrophage, and granulocyte-mediated defense mechanisms.[3, 4] This study was performed to characterize and quantitate lymphocytes and phagocytic cells in the vaginal environment and to determine the effects of lavage fluids on lymphocyte proliferation, macrophage phagocytosis, cell viability, and the expression of cell surface molecules on lymphocytes and macrophages, which are important in immunologic functions.

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Material and methods

Vaginal lavage fluid collection. Five healthy women of reproductive age (range 24 to 37 years) volunteered for the study. Three were in monogamouos sexual relationships and were taking oral contraceptives or using barrier methods of contraception. The other two women were sexually inactive and were not using any method of contraception. Vaginal lavage fluids were obtained at weekly intervals through three consecutive menstrual cycles. Women collected their own samples with an adapted 20 ml syringe (extra holes drilled at end for ease in aspiration). The syringe was filled with sterile Hanks' balanced salt solution and was inserted into the vagina with the woman in a supine position. The solution was expelled and drawn into the syringe

three times, then emptied from the syringe into a 50 ml sterile test tube and delivered to the laboratory within 1 hour of collection. Samples containing <15 ml of fluid were not included in the study.

Vaginal lavage fluid preparation. Vaginal lavage fluids were centrifuged at 600 *g* for 10 minutes. The supernatant was filtered through a 0.22 μm Millex filter (Millipore Corp., Bedford, Mass.) and aliquots were stored at −70° C. For some experiments unfiltered aliquots of supernatant were incubated at 37° C overnight to promote growth of vaginal flora. The cell pellet was resuspended in 1 ml phosphate-buffered saline solution and 20 μl was transferred to a hemocytometer where cells of white blood cell morphologic type and size (5 to 15 μm) were counted. Cell viability was assessed by trypan blue exclusion. A smear of the cell suspension was made on a glass microscope slide and stained with eosin-thiazine (Hemacolor, EM Diagnostics, Gibbstown, N.J.).

In those patients with $>2 \times 10^4$ white blood cells per sample, cell smears were made on Teflon-coated slides for immunohistologic assessment of white blood cells subpopulations as previously described.[5]

Monoclonal antibodies. Various monoclonal antibodies to white blood cells subpopulations were used in the immunoperoxidase studies: pan-leukocyte (anti-HLe-1, Becton Dickinson, Mountain View, Calif.), cytotoxic/suppressor T lymphocytes (Leu-2a, Becton Dickinson), helper/inducer T lymphocytes and some monocytes/macrophages (Leu 3a + b, Becton Dickinson), pan-T cell (Leu 4a + 5b, Becton Dickinson), macrophages (Dako-macrophage, Dako Corporation, Santa Barbara, Calif.), B lymphocytes, (Pan-B, Dako Corp.), and natural killer cells (NKH-1, Becton Dickinson).

The following monoclonal antibodies to immunologically relevant lymphocyte/macrophage surface antigens were used in radioimmunoassay studies of antigen modulation by soluble vaginal fluid factors: HLA-A,B,C, HLA-DR, interleukin-2 receptor (Il-2R), and anti-CD4 (Leu 3a+b); all of these were from Becton Dickinson.

Immunoperoxidase technique. A streptavidin-biotin-peroxidase system (Histostain—SP kit, Zymed Laboratories, San Francisco) was used as previously described.[5] Briefly, acetone-fixed eight-spot Teflon slides were thawed and air-dried. Nonspecific binding sites were blocked by nonimmune rabbit serum (10 minutes). Appropriately diluted monoclonal antibodies (listed previously) or phosphate-buffered saline solution (negative control) were incubated on individual spots of eight-spot slides for 30 minutes at 37° C. Slides were washed, and a Histostain-SP kit with a biotinylated secondary antibody was used to visualize bound antibodies. Slides were counterstained with hematoxylin, and coverslips were mounted with glycerol–polyvinyl alcohol aqueous mounting medium.

Phagocytosis. Macrophage phagocytosis of fluorescent carboxylate microspheres (Polysciences, Inc., Warrington, Pa.) was determined as previously described.[6] Briefly, human peripheral blood mononuclear cells were isolated by Ficoll-Hypaque centrifugation, washed three times, and resuspended at a concentration of 1×10^6 cells per milliliter in RPMI 1640 tissue culture medium buffered with sodium bicarbonate and containing 10% fetal calf serum, 10 mmol/L L-glutamine, and 10 mmol/L penicillin-streptomycin (GIBCO, Grand Island, N.Y.). Macrophages were obtained by incubation of a suspension of 1 ml of peripheral blood mononuclear cells on 12 mm round glass coverslips (Rochester Scientific Co., Inc., Rochester, N.Y.) in 24-well tissue culture plates (Falcon, Becton Dickinson, Lincoln Park, N.J.) for 1 hour at 37° C followed by removal of nonadherent cells by gentle pipetting and aspiration. Macrophages were cultured overnight in wells containing 1 ml of vaginal lavage fluid, 10% seminal plasma, 10% saliva, 1% nonoxynol 9 ($C_{33}H_{60}O_{10}$; Ortho Pharmaceutical Corporation, Raritan, N.J.), or Hanks' balanced salt solution (control) and 1 ml tissue culture medium. Microspheres were added and incubation was continued for 4 hours, after which the cells were vigorously washed to remove nonadherent microspheres and recultured for an additional hour in tissue culture medium to reduce background binding of microspheres to cells. The coverslips with attached cells were rinsed in Hanks' balanced salt solution, fixed in acetone for 10 minutes, and then placed cell side up on glass slides. Coverslips (24 × 50 mm) were mounted over cell monolayers with 80% glycerol and phosphate-buffered saline solution. Macrophage phagocytosis (percent of macrophages with more than five intracellular microspheres) was assessed on a Zeiss (Carl Zeiss, Inc., Thornwood, N.Y.) phase/epifluorescence microscope.

Radioimmunoassay. Human peripheral blood mononuclear cells (7×10^6) were incubated in 1 ml undiluted vaginal lavage fluid, in 10% or 1% seminal plasma, 10% saliva, or 0.01% nonoxynol-9 diluted in Hanks' balanced salt solution, or in Hanks' balanced salt solution alone at 37° C for 1 hour. The cells were then washed three times in RPMI 1640 medium and resuspended at a concentration of 2×10^6 cells per milliliter in RPMI medium containing 1% bovine serum albumin (washing solution); 0.2 ml of cell suspension was than added in triplicate to individual wells of 96-well, round-bottom microtiter plates (Linbro Division, Flow Laboratories, Inc., McLean, Va.). Cells were pelleted by centrifugation (500*g* for 5 minutes), the supernatant was removed from each well, and 0.02 ml of appropriately diluted monoclonal antibodies was added

Table I. Numbers and types of white blood cells in vaginal lavage fluid from healthy women (mean ± SD)

Phase of cycle	*Total leukocytes (CD45)*	*Granulocytes*	*Macrophages*	*T lymphocytes (CD2, CD3)*
Menstruation (n = 15)	$6.5 \times 10^4 \pm 2.3 \times 10^4$	$3.2 \times 10^4 \pm 8.8 \times 10^3$	$3.2 \times 10^4 \pm 2.5 \times 10^4$	$6.1 \times 10^3 \pm 2.6 \times 10^4$
Proliferative (n = 15)	$5 \times 10^3 \pm 3.6 \times 10^4$	$2 \times 10^3 \pm 3.3 \times 10^3$	$2.5 \times 10^3 \pm 3.5 \times 10^3$	ND
Periovulatory (n = 15)	ND	ND	ND	ND
Secretory (n = 15*)	ND	ND	ND	ND

ND, None detected ($<2 \times 10^4$ white blood cells per milliliter).
*Excludes the samples from two women with yeast vaginitis.

to the wells. After 1 hour of incubation at 4° C, the cells were washed three times and 200,000 counts/min of iodine 125–labeled rabbit antimouse immunoglobulin G (New England Nuclear, Boston) was added and incubated for an additional hour; then the cells were washed five times and counted in a γ-counter.

Lymphocyte proliferation. Lymphocyte proliferation was determined as previously described.[7] Briefly, peripheral blood mononuclear cells were adjusted to a concentration of 2.5×10^6 cells per milliliter in tissue culture medium. Cultures were set up in quadruplicate in 96-well tissue culture plates with each well containing 2.5×10^5 cells in 0.1 ml tissue culture medium; either 0.09 ml undiluted vaginal lavage fluid, 10% or 1% seminal plasma, 10% saliva, 0.01% nonoxynol-9, or Hanks' balanced salt solution; and 0.01 ml concanavalin A (Sigma Chemical Co., St. Louis; 30 gm/ml) or tissue culture medium. For the study of effects of pH on lymphocyte proliferation, tissue culture medium was made with RPMI 1640 that did not contain sodium bicarbonate and was pH adjusted with 0.1 mol/L phosphate buffer (pH range 3 to 12). Cultures were incubated for 72 hours at 37° C in a humidified atmosphere of 5% carbon dioxide and 95% air.

Lymphocyte proliferation was assessed by adding 0.5 μCi of [^{3}H]thymidine (13.1 Ci/mmol, New England Nuclear) 8 hours before harvesting with a MASH II (Microbiological Associates, Los Angeles) automatic sample harvester. Peripheral blood mononuclear cell viability was assessed before harvesting by trypan blue exclusion. Cells on dried glass fiber filter paper disks were suspended in 2 ml Betafluor scintillation cocktail (National Diagnostics, Somerville, N.J.), and radioactivity was measured in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.) (efficacy of the system 45%).

Statistical analysis. The unpaired Student t test and analysis of variance were used to calculate the significance of differences between test solutions. Statistical significance was assumed when the null hypothesis could be rejected with reasonable assurance ($p < 0.05$).

Results

Vaginal samples collected during menses and the proliferative phase of the menstrual cycle from healthy women contained numerous macrophages and granulocytes but few T lymphocytes, B lymphocytes, or plasma cells (Table I). Fewer than 10^4 leukocytes were found in samples from the periovulatory and secretory phases of the menstrual cycle, except in two women during one cycle each in which a symptomatic vaginal yeast infection was noted at midcycle and during the secretory phase. In these samples 2 to 3 $\times 10^5$ vaginal white blood cells were recovered; the majority of cells were macrophages and granulocytes, although $CD4^+$ lymphocytes and interleukin-2 $receptor^+$ lymphocytes were also observed. Few ($<10^4$) $CD8^+$ cytotoxic/suppressor T lymphocytes and no B lymphocytes were found. Within 1 week of therapy with fungicidal cream, hyphae and white blood cells were no longer observed in the vaginal lavage fluids.

Peripheral blood mononuclear cells were used to study the effects of vaginal lavage fluids on cellular defense functions because insufficient numbers of vaginal cells could be recovered for such studies. Vaginal lavage fluids collected during various phases of the menstrual cycle did not adversely affect peripheral blood lymphocyte viability as measured by trypan blue exclusion after a 2-hour incubation. Alteration of pH in tissue culture medium by replacement of bicarbonate buffer with 0.1 mol/L phosphate buffer (pH range 3 to 12) likewise did not affect leukocyte viability after 2 hours of culture. Cell viability was also unaffected by 10% seminal plasma, 10% saliva, or postcoital vaginal secretions without nonoxynol-9. The vaginal spermicide nonoxynol-9, however, when tested alone at concentrations ranging from 10% to 0.01% or when present in certain postcoital vaginal lavages, significantly affected peripheral blood leukocyte viability after 2 hours of exposure (<10% viable).

The function of peripheral blood–derived macrophages, as assessed by the ability of adherent cells to phagocytose fluorescent latex beads, was not signifi-

Helper/inducer T lymphocytes (CD4)	*Cytotoxic/suppressor T lymphocytes (CD8)*	*Interleukin-2 receptor*	*B lymphocytes (CD22)*
$5.2 \times 10^3 \pm 9.9 \times 10^3$	$4.4 \times 10^3 \pm 1.3 \times 10^4$	$8.3 \times 10^3 \pm 1 \times 10^4$	ND
ND	ND	ND	ND
ND	ND	ND	ND
ND	ND	ND	ND

Table II. Effects of vaginal lavage fluid on immune cell surface antigen expression (counts per minute, mean ± SD)

Secretion added	*No.*	*Bovine serum albumin control*	*Class I major histocompatibility antigens*	*Class II major histocompatibility antigens*	*CD4*
Control					
Media	15	3,136 ± 1,485	12,239 ± 1,756	3,186 ± 668	3,561 ± 783
Saliva (1:10)	15	2,321 ± 723	10,566 ± 647	3,623 ± 302	3,227 ± 780
Seminal plasma (1:10)	5	3,285 ± 2,328	11,108 ± 1,179	3,745 ± 439	3,332 ± 117
Nonoxynol-9 (1:10)	5	1,172 ± 635	1,149 ± 123**	1,010 ± 232†	1,058 ± 576†
Vaginal lavage fluid					
Menstrual	15	1,928 ± 556	10,589 ± 756	5,320 ± 2,310	3,091 ± 632
Proliferative	15	2,069 ± 455	11,251 ± 653	3,139 ± 264	4,742 ± 2,043
Periovulatory	15	1,739 ± 471	10,341 ± 984	3,553 ± 691	3,228 ± 385
Periovulatory postcoital‡	5	4,248 ± 3,092	14,788 ± 2,341	4,648 ± 1,047	4,006 ± 659
Secretory	15	1,782 ± 302	10,875 ± 369	4,957 ± 673	3,568 ± 963

*$p < 0.005$.
†$p < 0.025$.
‡Contained nonoxynol-9.

cantly affected by vaginal lavage fluid, 10% seminal plasma, or saliva. Furthermore, macrophages from vaginal lavage fluids collected at both menstrual and periovulatory phases of the cycle could adhere to plastic and phagocytose latex beads. Likewise, macrophage phagocytosis was not significantly affected by low pH conditions. However, neither adherence nor phagocytosis was observed in macrophage cultures exposed to 0.01% nonoxynol-9 or postcoital vaginal lavage fluids containing nonoxynol-9, presumably because of the disruptive effect of this compound on cell membranes (data not shown).

Expression of class I (HLA-A,B,C) and class II (HLA-DR) major histocompatibility antigens and the CD4 antigen (human immunodeficiency virus type 1, [HIV-1] receptor) on peripheral blood mononuclear cells was not affected by exposure to precoital or postcoital vaginal lavage fluids, seminal plasma, or saliva. Nonoxynol-9, when tested alone at a concentration of 1%, significantly affected surface antigen expression of all three markers; however, various postcoital vaginal lavage samples containing nonoxynol-9 had no effect on surface antigen expression (Table II).

Data showing effects of vaginal lavage fluid on lymphocyte proliferation are presented in Table III. Vaginal lavage fluids collected during the periovulatory and menstrual phases significantly inhibited lymphocyte proliferation. Vaginal lavage fluid collected and processed within 4 hours after coitus also had a very strong suppressive effect on lymphocyte proliferation, presumably because of the presence of seminal plasma, which has a strong immunosuppressive effect in these assay systems.[8] Nonoxynol-9 at a 0.01% concentration almost completely abolished lymphocyte proliferation, and postcoital samples containing nonoxynol-9 were more immunosuppressive than postcoital samples without nonoxynol-9 (48% vs 63% of control, $p < 0.01$). Saliva at a final concentration of 10% had no effect on lymphocyte proliferation. Aliquots of vaginal lavage fluids that were incubated overnight at 37° C before use had an increased inhibitory effect on lymphocyte proliferation (Table III), suggesting that vaginal flora may contribute to the immunosuppressive effect. Lymphocyte proliferation was significantly reduced in pH-controlled medium when the pH was <5 (Fig. 1).

Comment

Vaginal protective mechanisms against pathogens include mucosal secretory immunity, cyclic menses, bioactive reproductive secretions, low pH, and metabolic

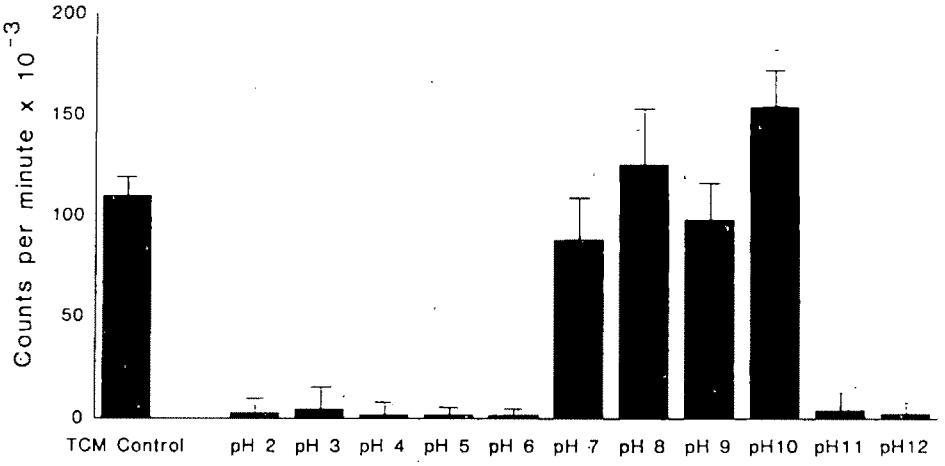


Fig. 1. Effects of pH on lymphocyte proliferation in concanavalin A mitogen assay.

Table III. Effects of vaginal lavage fluid on lymphocyte proliferation

Secretion added	*No.*	*Lymphocytes (counts/min, mean ± SD)*	*Proportion of control (%)*	*Significance*
Control				
Media	15	59,332 ± 1,483	—	—
Saliva (1:10)	15	56,738 ± 2,221	96	NS
Seminal plasma (1:100)	5	36,173 ± 4,111	61	<0.005*
Nonoxynol-9 (1:100)	5	12,332 ± 6,221	21	<0.0005
Vaginal lavage fluids				
Menstrual	15	45,630 ± 7,691	77	<0.025
Menstrual flora	15	34,256 ± 5,829	58	<0.005
Proliferative	15	58,541 ± 2,095	99	NS
Proliferative flora	15	43,167 ± 1,548	73	<0.0005
Proliferative postcoital	5	42,651 ± 3,217	72	<0.0005
Periovulatory	15	33,041 ± 10,788	56	<0.01
Periovulatory flora	15	31,171 ± 4,104	53	<0.0005
Periovulatory postcoital	5	37,243 ± 3,654	63	<0.005
Periovulatory postcoital with nonoxynol-9	5	28,325 ± 5,328	48	<0.005
Secretory	15	54,840 ± 7,315	92	NS
Secretory flora	15	35,789 ± 3,602	60	<0.0005
Secretory postcoital	5	38,421 ± 2,172	65	<0.005

NS, Not significant.

*Comparisons for statistical analysis were between treatment and media control groups.

products of commensal organisms.[9-12] Cellular mechanisms of host defense in the vagina have not been adequately described nor have white blood cells in the healthy human vagina been systematically studied in relation to the menstrual cycle. In this study vaginal lavage fluid was collected in five women on the first day of menses and thereafter every 7 days for three consecutive menstrual cycles. A total of 60 samples were studied. Our findings indicate that in healthy women appreciable numbers of white blood cells ($>2 \times 10^4$ white blood cells per milliliter) are recovered from the vagina only during menstruation and the proliferative phase of the menstrual cycle. The relatively low number of white blood cells ($6.5 \times 10^4 \pm 8.8 \times 10^3$) that were recovered from menstrual lavage fluids was not surprising because on average <80 ml of blood is lost during menses, and this occurs over a 3- to 5-day duration.[13] Menstrual lavage white blood cells consisted primarily of granulocytes and macrophages, which are abundant in the human endometrium.[14] Few T lymphocytes and almost no B lymphocytes were observed even in menstrual lavages. Some of the lymphocytes present, however, appeared to be activated as determined by interleukin-2 receptor positivity. The low numbers of white blood cells (primarily granulocytes and macrophages) observed in proliferative phase lavage fluid may represent residual leukocytes within the vagina after menses. We recently detected asymptomatic cervical-vaginal leukocytosis ($>10^4$ white blood cells) in 20% of healthy patients with infertility at mid-

cycle in an artifical insemination program and found an association between leukocytosis and cervical factor infertility.[15]

There was no discernible difference in vaginal cell counts or effects of lavage fluids in immune function assays between the women in this study. However, the small sample size precludes a definitive statement regarding effects of contraceptives or sexual activity on these parameters. To minimize the variable of acute white blood cells infiltration after insemination as a result of chemotactic factors and leukocytes in human semen,[16, 17] routine vaginal samples were collected after at least 48 hours of sexual abstinence. Postcoital samples were only tested in a few functional assays.

Cell-free vaginal lavage fluid from the menstrual and periovulatory phases of the cycle significantly inhibited concanavalin A–induced lymphocyte proliferation. Immunosuppressive effects of vaginal lavage fluids collected during menses and in the periovulatory phase of the menstrual cycle may indicate an attenuation of normal immune surveillance and enhanced susceptibility to infection during these times. Reports of an increased prevalence of endometritis-salpingitis and peritonitis as a result of *Neisseria gonorrhoeae* at the time of menses[18] support this speculation. Immunosuppression during the periovulatory phase may also be a mechanism for attenuation of a woman's immune response to spermatozoa. Data from this study indicate that lymphocyte proliferation is sensitive to low pH. The usual pH of the vagina in women of reproductive age is approximately 4; the lowest pH values are detected at midcycle during the periovulatory phase.[1] Therefore the low pH environment of the vagina may normally affect lymphocyte function, especially at midcycle. In our study vaginal samples were mixed with biocarbonate-buffered tissue culture medium (pH 7.4) and therefore were no longer at their physiologic pH. Thus pH was not the factor responsible for the immunosuppressive effect of vaginal lavage fluid obtained during mensturation and the proliferative phase of the cycle. In addition to organic acids that lower the vaginal pH, other immunosuppressive components have been described to be present in vaginal secretions.[19]

Data from this study may also have relevance to better defining mechanisms of sexual transmission of the HIV-1.[20] $CD4^+$ lymphocytes and macrophages can host HIV-1.[20] Epidemiologic studies have not revealed an increased risk of human immunodeficiency virus transmission during one phase of the menstrual cycle as opposed to another; however, traumatic bleeding during intercourse has been shown to be a risk factor for male-to-female human immunodeficiency virus transmission.[21] Our finding of few $CD4^+$ lymphocytes and macrophages in vaginal fluid of healthy women throughout the menstrual cycle supports this epidemiologic observation and suggests that healthy women may have a low human immunodeficiency virus infection rate because of low levels of HIV-1 target cells in the vagina. Vaginal pH may also affect HIV-1 transmission. In this study we demonstrated that T lymphocyte activation is inhibited by low pH, a condition normally found in the vagina. Lymphocyte activation is associated with the production of HIV-1.[22] Free HIV-1 is also rapidly inactivated by low pH.[23] Vaginitis often causes an elevation in vaginal pH.[24] Therefore women with vaginal infections may be at higher risk for HIV-1 acquisition and transmission because of two factors: increased numbers of infected-infectable cells and a more hospitable pH for infection.

Cell viability, macrophage phagocytosis, and surface expression of class I and class II major histocompatibility antigens, interleukin-2 receptor, and CD4 antigens were unaffected by vaginal lavage fluids except in the presence of the spermicide, nonoxynol-9, which was toxic to lymphocytes and macrophages. This is not surprising, because this compound is a detergent and disrupts the cell plasma membranes. Our finding that nonoxynol-9 interfered with the detection of cell-associated major histocompatibility complexes and CD4 surface antigens by radioimmunoassay when added directly to peripheral blood lymphocyte cultures at a concentration of 0.01%, but not when present in postcoital vaginal lavage fluid, probably reflect differences in spermicide concentration. Nonoxynol-9 inhibits HIV-1 replication in vitro[25] and in our study was demonstrated to be toxic to HIV-1 host cells ($CD4^+$ lymphocytes and macrophages) and to affect expression of the HIV-1 receptor CD4. Therefore vaginal application of high concentrations of nonoxynol-9 may significantly interfere with certain mechanisms underlying the sexual transmission of human immunodeficiency virus.

In conclusion, relatively few white blood cells were recovered from vaginal lavage fluid of healthy women except at menses when a moderate number of granulocytes and macrophages were observed. Lymphocyte but not macrophage defense functions were inhibited by low pH, which is the normal physiologic condition of the vagina, and cell-free vaginal lavage fluid collected during menses and at midcycle inhibited lymphocyte activation and proliferation. These observations suggest that lymphocyte activity may be inhibited at certain stages of the cycle but that phagocytes probably play an active role in host defense in the normal vaginal environment. Furthermore, the principal spermicide, nonoxynol-9, was toxic to both lymphocytes and macrophages and significantly inhibited their function. Further studies are needed to characterize macrophage, dendritic cell, and lymphocyte populations in the vag-

inal epithelium and regional lymph nodes that also may play a role in host defense and in HIV-1 transmission. Further work is also needed to determine what effects various infections have on the vaginal environment and the recruitment of white blood cells to this site.

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Effect of endothelium-derived relaxing factor inhibition on the umbilical-placental circulation in fetal lambs in utero

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OBJECTIVE: The purpose of this study was to examine whether basal endothelium-derived relaxing factor release contributes to regulation of resting umbilical-placental vascular resistance.
STUDY DESIGN: Because N^{ω}-nitro-L-arginine selectively inhibits the synthesis of nitric oxide, a major endothelium-derived relaxing factor, we investigated the effects of N^{ω}-nitro-L-arginine on umbilical-placental vascular resistance in 10 fetal lambs in utero. We inserted catheters and fitted an umbilical artery electromagnetic flow transducer around the common umbilical artery to measure umbilical blood flow and catheterized the left umbilical arterial hypogastric branch to allow selective umbilical-placental infusion (60 minutes) of pH-matched saline solution (control) or N^{ω}-nitro-L-arginine.
RESULTS: In seven normal fetal lambs, N^{ω}-nitro-L-arginine increased umbilical-placental vascular resistance and arterial pressures and decreased umbilical blood flow ($p < 0.05$); percentage changes from baseline were 50.8% ± 18.3%, 40.3% ± 8.1%, and −9.9% ± 6.4%, respectively. In three mildly asphyxiated (compromised) fetuses, these changes were 101.4% ± 28.7%, 31.2% ± 4.8%, and −37.9% ± 12.0%.
CONCLUSION: These data support the hypothesis that the basal endothelium-derived relaxing factor release plays a role in regulating resting umbilical-placental vascular resistance. (AM J OBSTET GYNECOL 1992;166:727-34.)

Key words: Nitric oxide, N^{ω}-nitro-L-arginine, placental circulation, umbilical vascular tone, umbilical vascular resistance

The umbilical circulation receives about 40% of the combined ventricular output in near-term fetal lambs.[1] Normal fetal growth and viability depend on the umbilical circulation for the supply of nutrients and exchange of gases. Any impairment in this circulation may increase the possibilities of growth retardation, fetal distress, and intrauterine fetal death.

The umbilical-placental vascular bed represents a vast network of blood vessels without neural supply, so that umbilical blood flow is predominantly controlled by general circulatory effects, such as systemic blood pressure, or locally produced humoral vasoactive substances.[2] Several vasoactive substances, including bradykinin, prostacyclin, and histamine, have been shown to affect the umbilical circulation.[3-5] However, relatively little is known about the effects of another potent vasoactive substance, endothelium-derived relaxing factor (EDRF), on the umbilical circulation.[6-9] Recently, it has been proposed that EDRF, or one of its components, could be nitric oxide, originating from endothelial cell metabolism of L-arginine.[10, 11] EDRF plays an important role in regulating pulmonary and systemic vascular resistances by stimulating soluble guanylate cyclase and subsequently increasing guanosine 3′,5′-cyclic monophosphate levels in adjacent vascular smooth muscle cells to elicit a vasorelaxant effect.[10-13] We therefore considered that EDRF could also play an important role in regulating umbilical vascular tone. To test this hypothesis, we used N^{ω}-nitro-L-arginine, which has been demonstrated to be a potent inhibitor of the enzymatic synthesis of nitric oxide from L-arginine.[14, 15] After an infusion of N^{ω}-nitro-L-arginine directly into the umbilical circulation through a hypogastric branch of the left umbilical artery, we evaluated the hemodynamic changes in fetal lambs in utero to determine whether basal production and release of EDRF plays a role in maintaining the low vascular tone in the intact umbilical-placental circulation.

Material and methods

Surgical preparation. Ten mixed Western fetal sheep of 133 to 137 days' gestation (term 145 days) were studied. Before surgery, the ewe fasted for 24 hours and water was withheld overnight. Low epidural anesthesia was achieved with 4 ml 1% tetracaine hydrochloride. To maintain light sedation, 50 to 100 mg ketamine hydrochloride was administered frequently. For adequate analgesia the fetus and ewe also received local anesthesia with 2% lidocaine hydrochloride for each

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Table I. Blood gases, pH, hemoglobin, and oxygen saturation before and after 60-minute infusion of normal saline solution in fetal lambs

	Before	*After*
Normal fetal lambs (n = 7)		
pH	7.36 ± 0.02	7.34 ± 0.03
P_{CO_2} (torr)	53.3 ± 3.6	54.2 ± 5.5
P_{O_2} (torr)	22.2 ± 1.3	21.8 ± 2.1
Oxygen saturation (%)	54.0 ± 4.8	54.0 ± 6.5
Hemoglobin (gm/dl)	10.7 ± 1.0	10.5 ± 0.9
Compromised fetal lambs (n = 3)		
pH	7.27 ± 0.02	7.25 ± 0.04
P_{CO_2} (torr)	67.3 ± 5.2	68.2 ± 4.4
P_{O_2} (torr)	15.3 ± 4.2	15.8 ± 2.8
Oxygen saturation (%)	32.3 ± 13.2	31.2 ± 11.5
Hemoglobin (gm/dl)	10.8 ± 2.4	11.1 ± 2.5

Values are means ± SD.

skin incision. Polyvinyl catheters were inserted into the maternal pedal artery and vein to monitor blood gases and pressure and to administer 500 to 1000 ml 10% dextrose in 0.9% saline solution at a rate of 3 to 5 ml/min during surgery and to administer antibiotics subsequently.

With aseptic techniques, a midline incision was made and the pregnant horn of the uterus was palpated and exposed. On opening the uterus, two 3.5F multiple-side-hole polyvinyl catheters were introduced into a distal cotyledonary branch of the umbilical vein and each was advanced to one of the main umbilical veins. Then the fetal hind limbs were exteriorized through the same uterine incision, and polyvinyl catheters were inserted into one pedal vein and artery in each hind limb and advanced to lie in the inferior vena cava and distal abdominal aorta, respectively. The hind limbs were extracted further to expose the lower abdomen and left flank. Then, as we have described in detail,[16] a left flank incision was made parallel to the spine, extending 3 to 4 cm cephalad from the left iliac crest. After blunt retroperitoneal dissection, the lower abdominal aorta, left iliac artery, common umbilical artery, left umbilical artery, and hypogastric (posterior pelvic) branch of the left umbilical artery were visualized clearly. The hypogastric branch was catheterized with a small polyvinyl catheter (inner diameter 0.030 inch). The catheter's tip was positioned near the origin of the left umbilical artery from the common umbilical artery. A precalibrated electromagnetic flow transducer of appropriate size was fitted around the common umbilical artery to measure umbilical blood flow.[16] The incision was closed in layers, and the fetus was returned to the uterus. A 12F polyvinyl catheter was placed in the amniotic cavity before the uterine incision was closed, and 100 mg gentamicin sulfate and 1,000,000 U penicillin G potassium were administered into the amniotic cavity. Warmed normal saline-solution was infused to replace lost amniotic fluid; then the uterine incision was closed and oversewn to prevent leakage of amniotic fluid.

All vascular catheters were flushed with 1 ml heparin sodium solution (1000 U/ml) to keep them patent and then sealed with special plugs. The catheters and transducer cable were exteriorized through the abdomen to a Teflon cloth pouch on the left flank of ewe. The labarotomy was closed in layers. One hundred milligrams of gentamicin sulfate and 1,000,000 U penicillin G potassium were administered intravenously to the ewe.

Antibiotics were administered intravenously to the ewe and into amniotic cavity daily. The preparation was approved by the Committee on Animal Research, University of California at San Francisco.

Experimental procedures. Experiments were performed on the second to fourth postoperative days with the ewe in the nonfasted state while the ewe stood quietly in a study cage with free access to alfalfa pellets and water. To determine the effects of N^{ω}-nitro-L-arginine on the umbilical circulation, two sequential series of experiments were performed in 10 fetal lambs, one during infusion of control solution (pH-matched normal saline) and the other during infusion of N^{ω}-nitro-L-arginine. We recorded the variables during four sequential steps in each animal in this order: (1) at baseline 1, (2) after control solution infusion for 1 hour, (3) at baseline 2, and (4) after N^{ω}-nitro-L-arginine infusion for 1 hour. We measured the fetal systemic arterial and venous pressures, umbilical venous pressure, umbilical blood flow, amniotic cavity pressure, heart rate, arterial blood gases and pH, hemoglobin, and oxygen saturation during each experimental condition. Before starting the infusion of either control or N^{ω}-nitro-L-arginine solution, we assured that flow and blood gases were in a steady condition for at least 30 minutes. At the end of study, positions of all catheters and the flow transducer were checked at autopsy.

Hemodynamic measurements. Strain-gauge transducers (model P23Db, Statham Instruments, Oxnard, Calif.) were used for continuous recording of aortic, umbilical venous, inferior vena caval, and amniotic fluid pressures. Mean pressure was obtained by electrical integration, and heart rate was obtained by a Beckman type 9857B cardiotachometer triggered from the arterial pressure pulse tracing. Umbilical blood flow was measured with a Statham SP 2202 electromagnetic flowmeter (frequency response flat to 100 Hz; 5% error; 2% reproducibility of flow measurement). During the study, pressures, heart rate, and umbilical blood

Table II. Hemodynamic variables before and after 60-minute infusion of normal saline solution in fetal lambs

	Before	*After*
Normal fetal lambs (n = 7)		
Umbilical vascular resistance (mm Hg/ml/min/kg)	0.027 ± 0.005	0.027 ± 0.006
Systemic arterial pressure (mm Hg)	47.6 ± 4.8	48.4 ± 4.4
Umbilical blood flow (ml/min/kg)	168 ± 16.9	167 ± 15.8
Δ (SAP − UVP) (mm Hg)	40.3 ± 4.1	40.1 ± 4.1
Heart rate (beats/min)	174 ± 14.6	172 ± 11.7
Compromised fetal lambs (n = 3)		
Umbilical vascular resistance (mm Hg/ml/min/kg)	0.029 ± 0.008	0.031 ± 0.009
Systemic arterial pressure (mm Hg)	41.3 ± 5.0	42.3 ± 4.7
Umbilical blood flow (ml/min/kg)	138 ± 31.6	134 ± 28.0
Δ (SAP − UVP) (mm Hg)	34.7 ± 4.2	36.0 ± 4.4
Heart rate (beats/min)	177 ± 21.6	174 ± 19.7

Values are means ± SD. Δ *(SAP − UVP)*, Difference between mean systemic arterial and umbilical venous pressures.

flow were recorded on a Beckman eight-channel Dynograph direct-writing recorder. All fetal pressures were corrected to amniotic fluid pressure as a zero reference level. Umbilical-placental vascular resistance was calculated from umbilical blood flow measured by the precalibrated electromagnetic flow transducer, and mean pressures were measured in the umbilical vein and descending aortic simultaneously. We assumed no significant difference between descending aortic and umbilical arterial pressures. Fetal blood samples were obtained from the descending aorta for measurement of pH, P_{CO_2}, and P_{O_2} (model 158 pH/blood gas analyzer, CIBA Corning, Medfield, Mass.) and of hemoglobin concentration and hemoglobin oxygen saturation (model OSM 2 hemoximeter, Radiometer, Copenhagen) during each experimental condition.

Radioactive microsphere analysis. To revalidate the accuracy of umbilical blood flow measurements by the electromagnetic transducer method, we simultaneously measured umbilical blood flow 25 times in eight fetal lambs with radionuclide-labeled microsphere injections during periods when the flowmeter was recording a steady umbilical blood flow. In all instances microspheres labeled with cobalt 57, chromium 51, gadolinium 153, indium 114, manganese 54, niobium 95, tin 113, strontium 85, and zinc 65, selected at random, were injected into the inferior vena cava over 15 to 20 seconds. A reference blood sample was withdrawn from the fetal descending aortic catheter with a constant pump rate of about 4 ml/min, beginning immediately before injection of microspheres and continuing for at least 40 seconds after the injection had been completed. The total volume of blood removed for sampling was calculated, and an equal volume of fetal or maternal blood was replaced after the microsphere study.

On completion of the last experiment, the ewe was killed with an overdose of pentobarbital. The uterus and placenta were dissected, fixed in formalin, and then carbonized. The radioactivities of placental tissue and of the reference blood samples were counted with a well-type γ-scintillation counter and a 1000-channel pulse-height analyzer (Norland, Fort Atkinson, Wis.) Umbilical blood flow was calculated as previously described.[17]

Table III. Blood gases, pH, hemoglobin and oxygen saturation before and after 60-minute infusion of Nω-nitro-L-arginine in fetal lambs

	Before	*After*
Normal fetal lambs (n = 7)		
pH	7.35 ± 0.02	7.34 ± 0.02*
P_{CO_2} (torr)	53.7 ± 4.4	55.5 ± 4.4*
P_{O_2} (torr)	22.3 ± 1.7	21.4 ± 1.7
Oxygen saturation (%)	54.8 ± 4.7	52.8 ± 5.2*
Hemoglobin (gm/dl)	10.4 ± 1.1	10.6 ± 1.0
Compromised fetal lambs (n = 3)		
pH	7.24 ± 0.02	7.19 ± 0.01*
P_{CO_2} (torr)	69.8 ± 8.1	72.1 ± 6.0
P_{O_2} (torr)	18.1 ± 4.7	15.7 ± 3.8
Oxygen saturation (%)	33.7 ± 11.7	31.6 ± 12.4*
Hemoglobin (gm/dl)	11.0 ± 3.2	12.1 ± 2.9*

Values are means ± SD.
*$p < 0.05$, between baseline and infusion observation.

Drug preparation and dosage. Immediately before the study, Nω-nitro-L-arginine (Sigma Chemical Co., St. Louis) was dissolved in normal saline solution at a concentration of 10 mg/ml and titrated to a pH of 2.8 to 3.0 by the addition of hydrochloric acid or sodium hydroxide to prevent precipitation. Each fetal lamb was given a 30 mg bolus of Nω-nitro-L-arginine followed by a 1.5 to 2.4 mg/kg/min infusion into the hypogastric branch of the left umbilical artery for 60 minutes. Previous experience in our laboratory has shown that the effects of EDRF on the pulmonary circulation could be inhibited by a 60-minute continuous infusion of Nω-nitro-L-arginine[18] and that the effects of Nω-nitro-L-ar-

Table IV. Hemodynamic variables before and after 60-minute infusion of N$^{\omega}$-nitro-L-arginine in fetal lambs.

	Before	*After*
Normal fetal lambs (n = 7)		
Umbilical vascular resistance (mm Hg/ml/min/kg)	0.026 ± 0.005	0.038 ± 0.010*
Systemic arterial pressure (mm Hg)	46.3 ± 3.1	64.9 ± 4.7*
Umbilical blood flow (ml/min/kg)	169 ± 18.2	153 ± 24.2*
Δ (SAP − UVP) (mm Hg)	38.4 ± 3.9	51.7 ± 4.5*
Heart rate (beats/min)	172 ± 12.5	150 ± 13.4*
Compromised fetal lambs (n = 3)		
Umbilical vascular resistance (mm Hg/ml/min/kg)	0.031 ± 0.011	0.061 ± 0.019*
Systemic arterial pressure (mm Hg)	41.7 ± 6.0	54.7 ± 8.1*
Umbilical blood flow (ml/min/kg)	134 ± 28.7	84 ± 24.1*
Δ (SAP − UVP) (mm Hg)	35.3 ± 5.1	43.3 ± 5.7*
Heart rate (beats/min)	187 ± 20.0	146 ± 14.0*

Values are means ± SD. Δ *(SAP − UVP)*, Difference between mean systemic arterial and umbilical venous pressures.
*$p < 0.05$, between baseline and infusion observation.

ginine were reversible by L-arginine infusion.[19] We followed this dosage to study the effects on the umbilical-placental circulation. To make sure that the acidity of the N$^{\omega}$-nitro-L-arginine solution alone produced no significant alterations in the fetal hemodynamic variables and blood gases, each fetal lamb received an equivalent infusion of normal saline solution that had been titrated to the same pH.

Statistical analysis. All data are presented as means ± SD. Hemodynamic variables and blood gas values during each experimental condition were compared with baseline data by paired t test. We also compared the percentage changes in variables between infusions of normal saline solution and N$^{\omega}$-nitro-L-arginine by paired t test. A probability of $p < 0.05$ was chosen to represent statistical significance.

Results

Data were obtained from 10 near-term fetal lambs in utero with a mean weight of 3.04 kg (range 2.42 to 3.58 kg). Before infusion of N$^{\omega}$-nitro-L-arginine or normal saline solution, blood gases and hemodynamic variables were checked. The fetuses were divided into two groups according to the blood gas data (Table I). Data in group 1 fetuses (normal, n = 7) were within normal limits and those in group 2 (compromised, n = 3) were not (pH < 7.3, P_{O_2} < 20 torr, P_{CO_2} > 60 torr).

After a 60-minute infusion of normal saline solution, there were no significant changes in hemodynamic variables, including umbilical blood flow and umbilical vascular resistance, in groups 1 and 2 (Tables I and II). However, selective intravascular infusion of N$^{\omega}$-nitro-L-arginine resulted in a decrease in pH and oxygen saturation in both groups and an increase in P_{CO_2} in group 1 (Table III). Hemoglobin increased in group 2.

After infusion of N$^{\omega}$-nitro-L-arginine, there were significant increases in umbilical vascular resistance, systemic arterial pressure, and the difference between mean systemic arterial and umbilical venous pressures, especially in compromised fetal lambs (Table IV). Umbilical blood flow and heart rate decreased in both groups.

We compared the percentage changes in major determinants of umbilical-placental circulation effected by infusions of normal saline solution of N$^{\omega}$-nitro-L-arginine solutions, respectively. The percentage changes of umbilical vascular resistance, systemic arterial pressure, and umbilical blood flow from baseline after infusion of N$^{\omega}$-nitro-L-arginine were 50.8% ± 18.3%, 40.3% ± 8.1%, and −9.9% ± 6.4% in group 1 and 101.4% ± 28.7%, 31.2% ± 4.8%, and −37.9% ± 12.0% in group 2. After infusion of normal saline solution, there were no significant changes. These results are shown in Figs. 1 to 3.

Measurements of umbilical blood flow derived from electromagnetic flow transducers and those derived from the radionuclide-labeled microsphere injections correlated very well (Fig. 4).

Comment

An EDRF was first reported by Furchgott and Zawadzki[20] in 1980. Over the past 10 years it has been shown that at least one form of EDRF is nitric oxide or a compound that releases nitric oxide.[10, 11] This increases guanosine 3′,5′-cyclic monophosphate concentration in vascular smooth muscle cells by activating the soluble isoenzyme form of guanylate cyclase. Subsequently, guanosine 3′,5′-cyclic monophosphate evokes the relaxant effect by activating guanosine 3′,5′-cyclic monophosphate–dependent protein kinase and dephosphorylation of myosin light chain. Hence EDRF clearly plays an important role in regulating vascular tone in the pulmonary and systemic circulations.[10-13, 21]

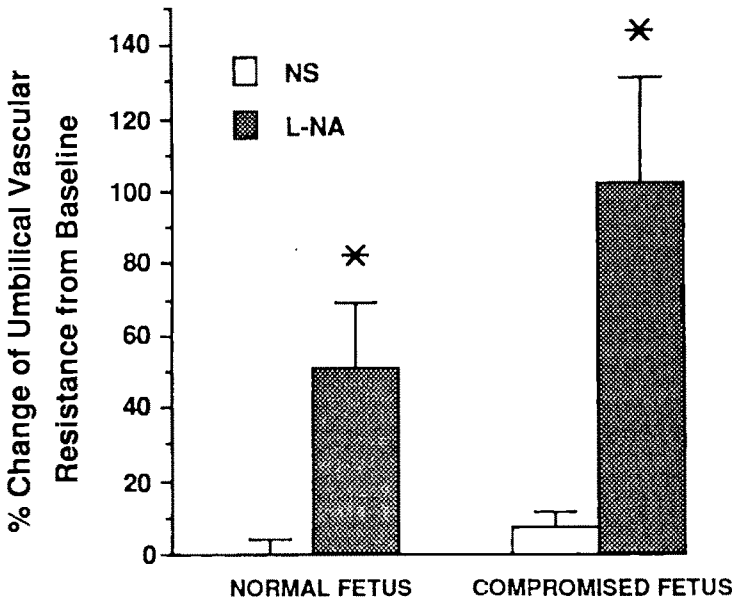


Fig. 1. Percent change in umbilical vascular resistance from baseline after infusions of normal saline solution *(NS)* and Nω-nitro-L-arginine *(L-NA)*, respectively. *Asterisk*, $p < 0.05$ Nω-nitro-L-arginine versus normal saline solution group.

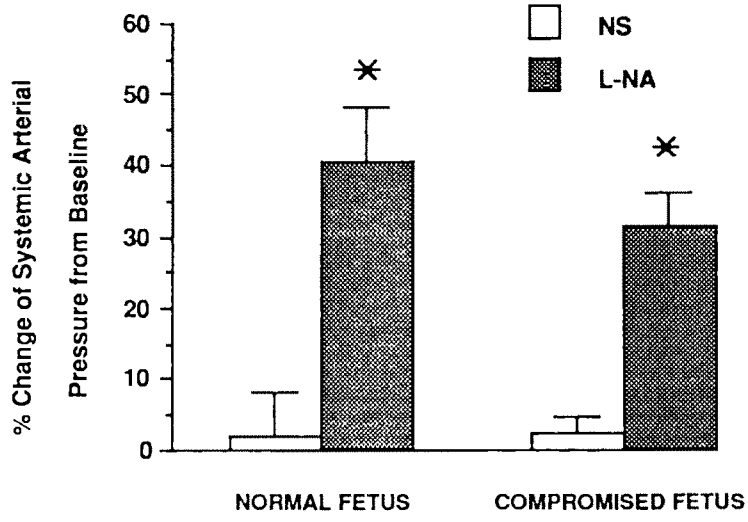


Fig. 2. Percent change in systemic arterial pressure from baseline after infusions of normal saline solution *(NS)* and Nω-nitro-L-arginine *(L-NA)*, respectively. *Asterisk*, $p < 0.05$, *(L-NA)* Nω-nitro-L-arginine versus normal saline solution group.

However, limited and contradictory information is available on the effects of EDRF on the umbilical circulation. Van de Voorde et al.[6] reported that production of EDRF from human umbilical arteries and veins was stimulated by histamine and adenosine triphosphate but not by acetylcholine. Subsequently, Buga et al.[22] reported that EDRF could be released from human umbilical veins. However, Griggs et al.[23] found that human placental chorionic plate arteries did not have the ability to decrease vascular tone in response to either A23187 or histamine, and Monuszko et al.[24] reported that umbilical vessel rings had no relaxant response to either A23187 or acetylcholine. Therefore they considered that these vessels lacked an endothelium-dependent relaxant capability. Recently, Chaudhuri et al.[8] and Myatt et al.[7] found that the vascular tone of precontracted human umbilical or placental arteries could be reduced by glyceryl trinitrate or S-nitroso-N-acetylpenicillamine. They therefore considered that basal release of nitric oxide might contribute to regulating the low

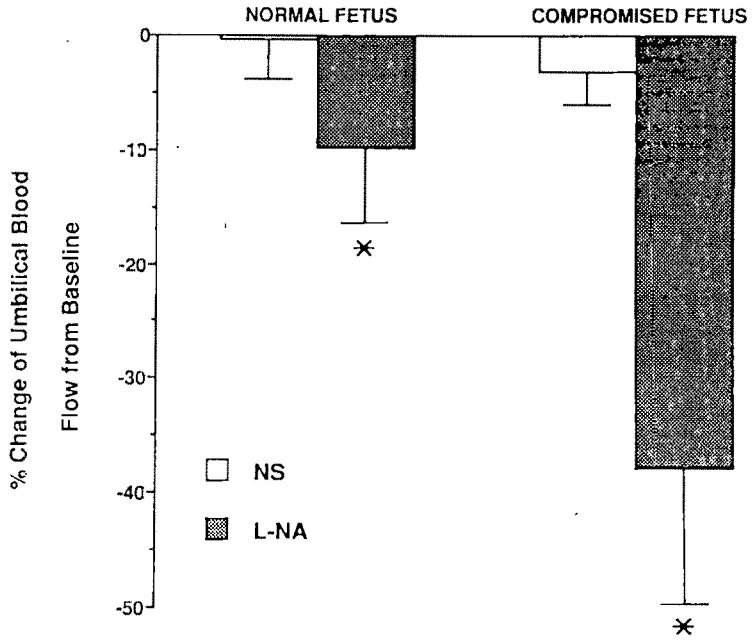


Fig. 3. Percent change in umbilical blood flow from baseline after infusions of normal saline solution *(NS)* and N$^{\omega}$-nitro-L-arginine *(L-NA)*, respectively. *Asterisk*, $p < 0.05$, N$^{\omega}$-nitro-L-arginine versus normal saline solution group.

resting umbilical vascular tone. A recent report by Chaudhuri et al.[9] may explain these differences because they showed that human umbilical arteries in vitro are less sensitive to EDRF than are chorionic plate arteries. It also is possible that the mechanism of stimulation of EDRF production differs between sites or even species. In this in vivo study we found that umbilical vascular resistance increased after a selective 60-minute infusion of N$^{\omega}$-nitro-L-arginine, an L-arginine analog that competitively inhibits the synthesis of EDRF from L-arginine. Although we did not demonstrate N$^{\omega}$-nitro-L-arginine specificity in these particular animals by reversing its effects with L-arginine, we have done this previously in newborn lambs[19] and also in a different group of fetal lambs (unpublished observations). These findings are consistent with those from the in vitro studies of Myatt et al.[7] and Chaudhuri et al.[8, 9] and show that basal release of EDRF might play an important role in maintenance of a low umbilical vascular resistance.

Autoregulation is an important physiologic mechanism for maintenance of constant organ blood flow with variations in arterial blood pressure. However, current evidence indicates that there is little autoregulation in the umbilical circulation.[25] Because the umbilical arteries are not innervated beyond their proximal segment, perfusion pressure is considered a major determinant of blood flow through the relatively passive umbilical circulation.[26] In this study, after infusion of N$^{\omega}$-nitro-L-arginine, both the systemic arterial pressure and calculated umbilical vascular resistance were increased and umbilical blood flow was decreased, in spite of the increase in perfusion pressure. This indicates that EDRF is normally produced in the resting fetal state and acts as an umbilical-placental vasodilator on a continuing basis, thereby regulating basal vascular tone.

We found that umbilical blood flows were markedly decreased by N$^{\omega}$-nitro-L-arginine infusion in compromised fetal lambs and that fetal condition deteriorated, whereas it was well tolerated in normal fetal lambs. This further suggests an important role for EDRF in maintaining umbilical-placental perfusion during periods of fetal stress. The stereospecific analog of L-arginine therefore appears to be a useful pharmacologic tool for further studies of basal or stress-related release of EDRF by the umbilical circulation.

Reliable information about the reactivity of the intact umbilical circulation to vasoactive drugs is scant because several factors may obscure or modify the responses during study. For example, umbilical vasospasm may be sustained during studies of acutely exteriorized animal models or in isolated perfused placental preparations.[27, 28] In addition, some investigators also found that when vasoactive drugs are infused into the inferior vena cava to evaluate the effects on the umbilical circulation, the systemic effects might obscure the true results. Parisi et al.[29] pointed out an unexpected result in their study. They found that administration of prostacyclin to the fetal lamb through the inferior vena cava apparently did not cause umbilical vasodilatation but

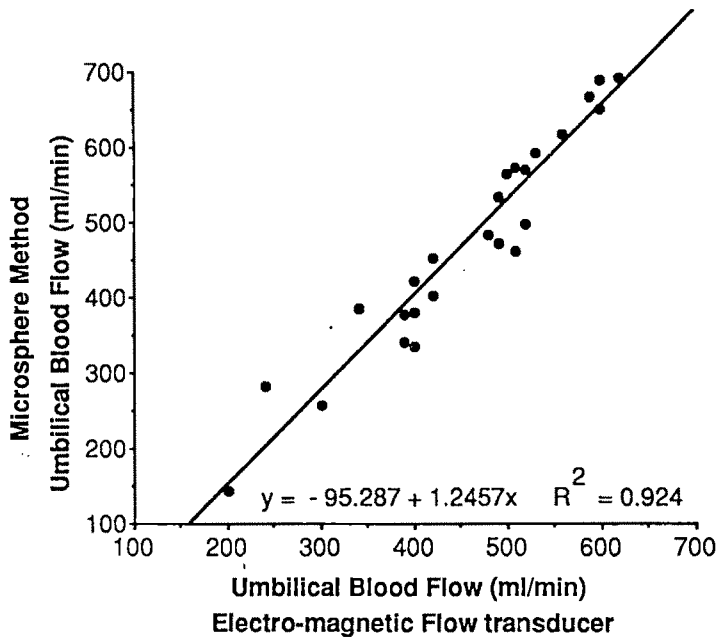


Fig. 4. Comparison of umbilical blood flow measurements derived from injections of microspheres and those recorded simultaneously by electromagnetic flow transducer.

caused vasoconstriction. This resulted from systemic and pulmonary vasodilatation shunting blood away from the placenta to the fetal lung. Therefore we used chronic unanesthetized preparations and infused Nω-nitro-L-arginine directly into the umbilical circulation to attempt to evaluate the true effects on the umbilical-placental circulation without additional stresses imposed and to avoid any possibility that these might modify the results. Although we allowed only a 2- to 4-day postsurgical recovery period, all baseline measurements in our normal group of animals were similar to those previously recorded by us and others in many different studies. We therefore consider that these studies were performed under relatively stress-free conditions.

In clinical situations maintaining an adequate umbilical circulation is essential for fetal growth. In the past, several authors have suggested that the synthesis of prostacyclin or EDRF by umbilical arteries of babies born to mothers after complicated pregnancies could be impaired.[30, 31] In addition, Pinto et al.[31] further considered that the production and release of EDRF by human umbilical vessels was inhibited because cytotoxic materials destroyed endothelial function and showed that the production of EDRF could not be restored by an infusion of superoxide dismutase or L-arginine.[31] Hence EDRF, together with prostacyclin, may play an important role in controlling platelet and leukocyte accumulation in the umbilical vascular wall and in regulating smooth muscle contraction to maintain adequate umbilical blood flow. Impairment of EDRF production could then result in intrauterine growth retardation.

In summary, the hemodynamic effects of Nω-nitro-L-arginine, a stereospecific L-arginine analog, on the umbilical-placental circulation in intact fetal lambs suggest that EDRF plays an important role in maintaining the normal low resistance in this circulation. We think impairment of EDRF production may contribute to umbilical vascular disorders, especially in babies born to preeclamptic mothers. Further studies may elucidate an important pathophysiologic mechanism for fetuses with intrauterine growth retardation or asphyxia without apparent reasons.

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GnRH Analogs: Optimal Management Strategies

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Nafarelin in the management of endometriosis: Quality of life assessment

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Quality of life is important when comparing the relative advantages of nafarelin versus danazol for the treatment of endometriosis. Recent studies have investigated the potential differences between the safety profiles of nafarelin and danazol and the impact of these profiles on the patient's quality of life. Results show that although these drugs have similar efficacy, they are associated with very different safety profiles. Most notable are the androgenic effects such as weight gain associated with danazol. With nafarelin, hypoestrogenic side effects, such as hot flashes, are more common. More important, these differences in safety profiles may prove to be relevant to patient satisfaction and compliance with therapy. (AM J OBSTET GYNECOL 1992;166:735-9.)

Key words: Quality of life, androgenic, hypoestrogenic

The efficacy of nafarelin acetate in reducing the size of endometriotic implants has been demonstrated repeatedly in controlled trials.[1-4] Nafarelin dosages of 400 μg daily have compared favorably with 800 mg of danazol daily when laparoscopic scores were compared.[1, 2] These studies, conducted in the United States and Europe, have also shown that nasally administered nafarelin reduced both estradiol and estrone levels to the range associated with medical castration. In contrast, orally administered danazol, although achieving reductions in circulating levels of these hormones, did not achieve medical castration. These findings provide evidence to suggest that although both medical therapies are effective against endometriosis, they are not interchangeable with respect to their safety profiles. Indeed, data suggest that important differences exist between nafarelin and danazol that are not directly related to the objective measurement of improvement in endometriotic implants. Whether the goal is treatment of infertility or relief of pain and other symptoms, a careful examination is needed of the potential risks posed by treatment of endometriosis. Clinical experience with danazol suggests that its efficacy is often achieved at a cost of an increased incidence of androgenic side effects, such as weight gain, acne, and hirsutism.[4-6] Thus an alternative therapy that is associated with either fewer or more tolerable side effects is of clinical interest.

Estimates of the number of women with endometriosis are confounded by the considerable proportion who remain without symptoms. A definitive diagnosis still requires direct visualization with laparoscopy or laparotomy. Nevertheless, it has been suggested that as many as 7% of women of reproductive age (15 to 45 years of age) are affected by endometriosis[7] and that the rate of clinical recurrence after discontinuation of therapy is high.[8] Common symptoms in women who consult a physician for possible endometriosis are dysmenorrhea, dyspareunia, dyschezia, and abnormal menstrual bleeding; however, the symptom responsible for most patient complaints is chronic pelvic pain. The persistence of pelvic pain can have a negative impact on physical and sexual function as well as a sense of well-being and other psychological aspects. The result may be a measurable decrease in the patient's quality of life.

The potential impact on quality of life on the patient has prompted comparisons between nafarelin and danazol. The intent of these investigations is to evaluate the efficacy of these agents in the context of quality of

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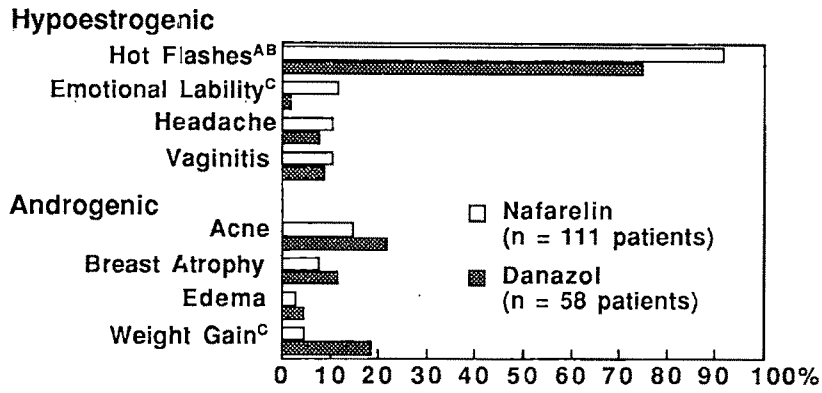


Fig. 1. Summary of most frequent adverse effects (probably related to therapy) in patients treated with nafarelin or danazol.[1] *A*, Women with hot flashes before treatment were excluded (five nafarelin; one danazol). *B*, $p < 0.01$ for between-treatment difference. *C*, $p < 0.05$ for between-treatment difference.

life variables using both objective and subjective measures of patient response. Danazol, administered orally in therapeutic doses of 800 mg daily, is a synthetic derivative of 17α-ethinyl testosterone that directly inhibits enzymatic synthesis of ovarian steroids and inhibits pituitary gonadotropin secretion. It is also highly androgenic partly because of perhaps increased androgen bioavailability that results from danazol binding to sex hormone–binding globulin.[9] This results in a substantial increase in free testosterone, which probably contributes to the androgenic side effects, such as hirsutism, lowering of voice, and especially weight gain, that are associated with danazol.

Nafarelin, administered as a nasal spray, is an analog or agonist of gonadotropin-releasing hormone (GnRH). When administered long term in dosages ranging from 400 to 800 μg daily, it paradoxically suppresses the release of luteinizing hormone and follicle-stimulating hormone, which in turn suppress gonadal production of estradiol and testosterone.[9] Both drugs deprive endometriotic implants of the gonadal steroids they need to remain viable.

Safety profiles

Nafarelin versus danazol. In the recent U.S. study,[1] 169 patients with laparoscopically confirmed endometriosis were enrolled in a multicenter, double-blind study. The randomization procedure assigned two of every three patients to receive nafarelin, 400 μg daily ($n = 111$), and one of three patients to danazol, 600 mg daily ($n = 58$). All patients were evaluated before treatment, 2 weeks after beginning treatment, monthly throughout the 6-month study period, and 1 month after therapy was stopped. Symptoms of endometriosis, such as pelvic pain, dyspareunia, and dysmenorrhea, were evaluated; pelvic examinations were performed; and all patients were asked to complete questionnaires designed to evaluate changes in quality of life indicators.

Of the patients randomized, six receiving nafarelin and three receiving danazol discontinued treatment because of adverse effects. An additional patient receiving danazol withdrew because of elevated liver enzyme levels. Twelve other patients were withdrawn for administrative reasons. A comparison of the 98 nafarelin-treated and 49 danazol-treated patients who completed the treatment phase of the study revealed that the two populations were well matched demographically. There was no statistically significant difference between the groups with regard to age, severity of clinical symptoms at admission, complaints of infertility, or history of pelvic inflammatory disease. The percentage of patients who desired pregnancy, pain relief, or both was also similar in the two groups.

The two therapies were similar in the magnitude of change in laparoscopic scores. Specifically, 77% of the nafarelin group and 83% of the danazol group had lower laparoscopic scores at the end of treatment compared with baseline scores. Similarly, equal efficacy was seen in the improvement in symptom scores. Patients reporting mild or no symptoms of endometriosis at the end of therapy comprised 96% of the nafarelin group and 92% of the danazol group. Among those who were reevaluated 6 months after the end of treatment, 79% of the nafarelin group and 75% of the danazol group reported mild or no symptoms.

Although the vast majority of patients found the side effects associated with the assigned therapy to be acceptable, approximately 5% of patients initially randomized to each study group discontinued therapy because of adverse events. Although the percentage of patients discontinuing therapy in the two groups was similar, the incidence of specific adverse effects differed substantially between the two groups.

The adverse effects associated with treatment could be divided into two categories: hypoestrogenic and androgenic effects (Fig. 1). As expected hypoestrogenic effects were more pronounced in nafarelin-treated pa-

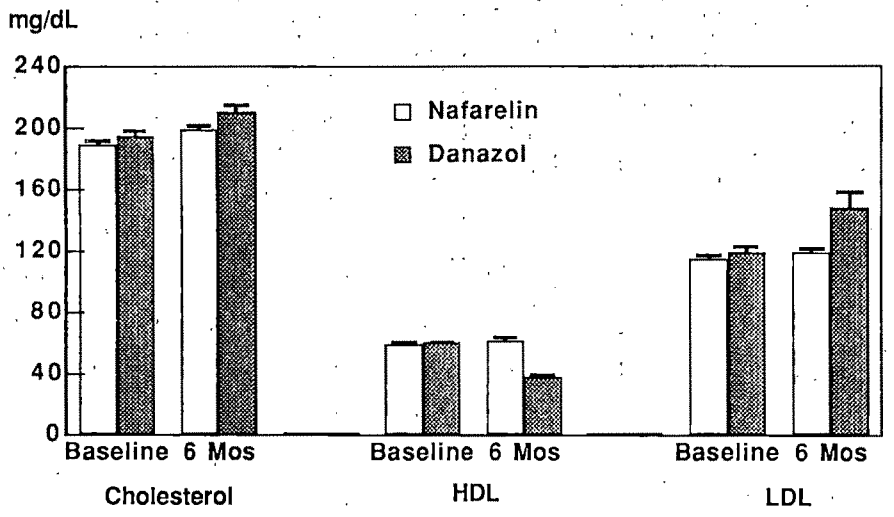


Fig. 2. Mean (±SE) lipid concentrations at baseline and after 6 months treatment with nafarelin or danazol for endometriosis.[1] *HDL,* High-density lipoprotein; *LDL,* low-density lipoprotein.

tients than in those receiving danazol. The incidence of hot flashes was 92% among those receiving nafarelin compared with 75% in patients receiving danazol ($p < 0.01$ for between-treatment differences). Emotional lability also was more common in the nafarelin group (12%) than among danazol-treated patients (2%; $p < 0.01$ for between-treatment differences). In addition, an increased incidence of headache and vaginal dryness (and vaginitis) was reported in the nafarelin group compared with danazol-treated patients. However, none of the nafarelin-treated patients discontinued therapy because of these symptoms.

Androgenic side effects were more marked among patients treated with danazol. The adverse effect responsible for most complaints was weight gain. In the U.S. study, 19% of patients in the danazol group complained of weight gain compared with only 5% in the nafarelin group ($p < 0.01$ for between-treatment differences). By the third month of treatment, there was a significant difference in weight between patients treated with danazol versus those given nafarelin. Moreover, no woman taking nafarelin showed an appreciable change in mean body weight throughout the 6-month study. Other androgenic adverse effects such as acne, breast atrophy, and edema tended to occur more frequently in danazol-treated patients. Myalgia, which was reported by 12% of danazol-treated patients, occurred in only 2% of those who received nafarelin. Also, seborrhea, hirsutism, and increased libido occurred more frequently in patients who received danazol.

The effects of nafarelin and danazol on lipid profiles were also evaluated. Results showed that danazol was associated with a significant ($p < 0.01$) decrease in high-density lipoproteins and an increase in low-density lipoproteins. In contrast, nafarelin significantly ($p < 0.01$) raised high-density lipoprotein levels, whereas low-density lipoprotein levels were unchanged (Fig. 2).[1] Deterioration of lipid profiles after danazol therapy may represent increased risk to the patient's cardiovascular profile.

Additionally, one patient in the danazol group and no patient in the nafarelin group discontinued therapy as a result of an abnormal liver function test. Patients treated with danazol have shown increases in alanine aminotransferase and creatine phosphokinase levels into the abnormal range; these effects are not observed with nafarelin.[10]

Quality of life evaluation. The quality of life measurements in the U.S. study were obtained by a questionnaire composed of 22 simple questions that comprised the Psychological General Well-Being Index plus a modification of Part II of the Nottingham Health Profile, which requires only a patient's visual qualification of psychological state rather than a numerical quantification. The psychological and general well-being score reveals types of psychological conditions such as anxiety, depressed mood, positive well-being, self control, general health, and vitality. The health profile looks into aspects of the patient's life such as work; the quality of work produced, self-image, ability to handle domestic concerns, social life, home life, sex life, outside activities, and quality of time spent away with the family.

The results of these questionnaires failed to reveal statistically significant differences between the entire treatment group. However, when those patients seeking relief of symptoms of endometriosis were evaluated separately from those seeking pregnancy only, a difference in scores was observed. For example, patients in both treatment groups who were seeking pregnancy began with and maintained a positive score for work-related quality of life. However, patients seeking pain relief started with a depressed work score that im-

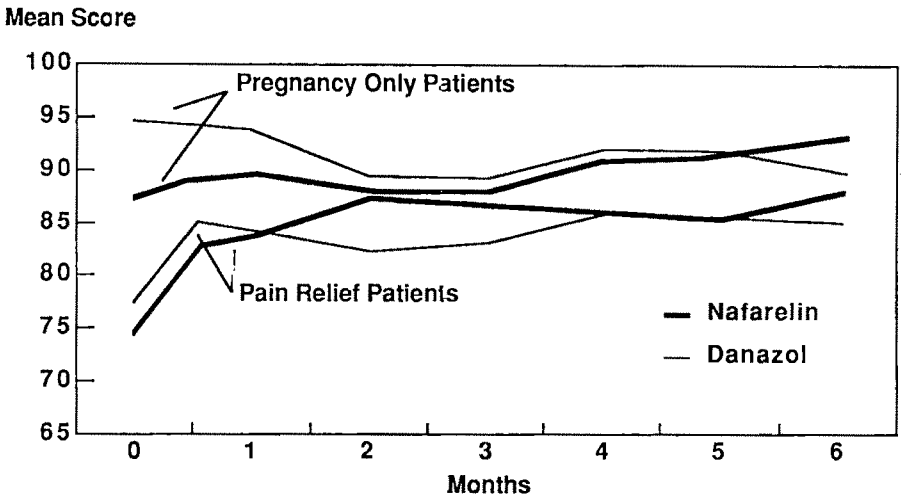


Fig. 3. Effect of treatment with nafarelin or danazol on quality of work scores.[1] Scores were stratified according to those seeking pain relief only or those seeking pregnancy.

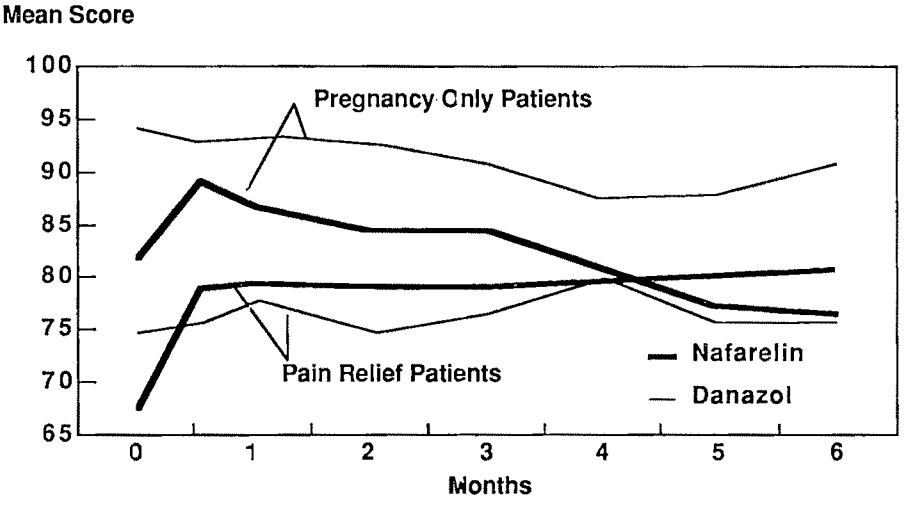


Fig. 4. Effect of treatment with nafarelin or danazol on the quality of sexual function.[1] Scores were stratified according to those seeking pain relief only or those seeking pregnancy.

proved during nafarelin but not during danazol treatment (Fig. 3). Although there were no between-group differences, overall, the nafarelin group showed significant ($p < 0.05$; paired t test) improvement from baseline in work productivity at all assessments, whereas there was no significant change in this measure in the danazol group.

The Nottingham Health Profile revealed another difference between the two treatment groups with respect to the quality of sexual function. Danazol-treated patients showed no significant improvement in quality of sexual function during treatment. Nafarelin-treated patients seeking pain relief had a lower mean score at baseline but did achieve and maintain a significant ($p < 0.01$; paired t test) improvement in quality of sexual function after 1 month of therapy (Fig. 4). The improved sexual function in this subgroup likely was the result of reduced pain in general and an improvement in dyspareunia. There was actually a decrease in the mean score over time among nafarelin-treated patients seeking pregnancy, however, this change was not statistically significant. Failure to show improvement in sexual function in the infertility subgroup, particularly in nafarelin-treated patients seeking pregnancy, was chiefly because of the complaint of vaginal dryness, an adverse effect associated with both nafarelin and danazol.

Comment

In the most recent U.S. study[1] and in past studies,[4-6, 11] nafarelin and danazol demonstrated similar efficacy for the treatment of endometriosis based on lap-

aroscopic scores. Thus the most important differences between these two agents may be their safety profiles and effect on the patient's perception of quality of life.

As has been shown in the past,[4-6, 12] most of the side effects attributed to danazol in this investigation were associated with its androgenic and anabolic properties. Although the 600 mg/day dose of danazol administered in this study is lower than the 800 mg/day typically administered for the management of endometriosis, the incidences of myalgia, weight gain, and acne still rose significantly above baseline levels and above incidences of these adverse effects in patients who received nafarelin. In contrast, the adverse effects associated with nafarelin appear to be associated primarily with hypoestrogenism.

A sensitive, qualitative instrument for measurement of quality of life in patients with endometriosis was difficult to find. Most instruments have been developed for an older population with chronic diseases. The quality of life profiles used in the U.S. study are generally not sensitive enough to differentiate between danazol and nafarelin. Scores of the Psychological General Well-Being Index improved significantly from baseline in both treatment groups. However, the Nottingham Health Profile scale did show that nafarelin was associated with a significant improvement in work-related indexes of quality of life that were not exhibited in the danazol group. Notably, when patients were grouped according to the goal of therapy, patients seeking pain relief showed an improvement in baseline quality of life measures, and the improvement seen with nafarelin on these measures was greater than with danazol.

More important, there is considerable evidence that nafarelin is comparable with danazol for the treatment of endometriosis. Because nafarelin exhibits a safety profile that is somewhat different from that of danazol, it represents an alternative to patients who cannot or are unwilling to tolerate danazol. Clinicians are more able to evaluate the relative risks of these two alternative therapies and to select an agent better suited to the goals and concerns of the individual patient.

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Hormone treatment of endometriosis: The estrogen threshold hypothesis

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In women with recurrent pelvic pain caused by endometriosis, hormonal therapy with a gonadotropin-releasing hormone agonist is an effective alternative to surgical therapy. The basis for medical treatment of endometriosis is that endometriosis lesions are dependent on estradiol for continued growth. Further, end organ tissue varies in its sensitivity to estradiol. This forms the basis of the estrogen threshold hypothesis, that is, that a concentration of estradiol that will partially prevent bone loss may not stimulate endometrial growth. Thus there is a hierarchy of organ response to estradiol such that calcium metabolism is most sensitive followed by gonadotropin secretion, vaginal epithelial growth, lipid metabolism, and liver protein production. Similarly, breast cancer is most sensitive and endometriosis is least sensitive to estrogen. These differences may allow the design of regimens with a gonadotropin-releasing hormone agonist that maintain a therapeutic response and ameliorate potential adverse effects. (AM J OBSTET GYNECOL 1992;166:740-5.)

Key words: Endometriosis, estradiol, gonadotropin-releasing hormone agonists, progestins

Endometriosis is the presence of tissue resembling endometrial glands and stroma outside the uterine cavity. Women with endometriosis typically present for medical care with one of three problems: an adnexal mass (endometrioma), infertility, or pelvic pain. For women with an endometrioma, surgical removal of the mass is both diagnostic and therapeutic. For women with stage III or stage IV endometriosis and infertility, surgical resection of endometriotic lesions and adhesions probably improves fecundity.[1,2] For women with pelvic pain, diagnostic laparoscopy may reveal the presence of endometriosis. At the time of initial surgical diagnosis, most clinicians will attempt to ablate the endometriosis with laser or electrosurgical techniques. Some clinicians would also advocate ablation, transection, or resection of the uterosacral ligaments at the time of initial surgical diagnosis. Many women with endometriosis and pelvic pain experience relief of pelvic pain for 3 to 12 months after such surgical procedures. However, in my experience, after such surgical procedures, most women will have a recurrence of pain within 12 months. For these women with recurrent pelvic pain caused by endometriosis, hormone therapy is an appropriate therapeutic choice and is usually very effective. The basis for hormone treatment of endometriosis is that endometriotic lesions are dependent on estradiol for their continued growth.

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Endometriosis is estrogen dependent

Clinical observations that suggest that endometriosis is sensitive to estrogen include the following: (1) Endometriosis is rare before puberty or after menopause; (2) surgical removal of both ovaries typically results in regression of endometriotic lesions; (3) production of a hypoestrogenic state with a gonadotropin-releasing hormone (GnRH) agonist such as nafarelin or leuprolide results in regression of endometriosis; and (4) endometriosis can develop in the prostatic utricle of men treated with diethylstilbestrol for prostate cancer.[3] Immunocytochemical studies indicate that virtually all endometriotic lesions contain estrogen receptors.[4] In experimental rat and human models of endometriosis, hypoestrogenism produces atrophy in endometriotic lesions, and estrogen administration causes the endometriotic lesions to grow.[5,6] In humans, the precise concentration of estradiol that will cause endometriotic lesions to atrophy or grow remains to be defined. However, estradiol concentrations in the range of 10 to 20 pg/ml typically result in the atrophy of endometriotic lesions.[7-10] Estradiol concentrations greater than 60 pg/ml are often associated with the growth of endometriotic lesions. Although estradiol concentrations in the range of 10 to 20 pg/ml result in the regression of endometriotic lesions, they also may be associated with loss of trabecular bone mineral, vasomotor symptoms, and atrophy of secondary sexual characteristics.[7-10] An important question is whether a circulating estradiol concentration in the range of 30 to 45 pg/ml might be associated with no growth of endometriotic lesions and minimal changes in bone mineral density. Preliminary evidence to support this concept is reviewed below.

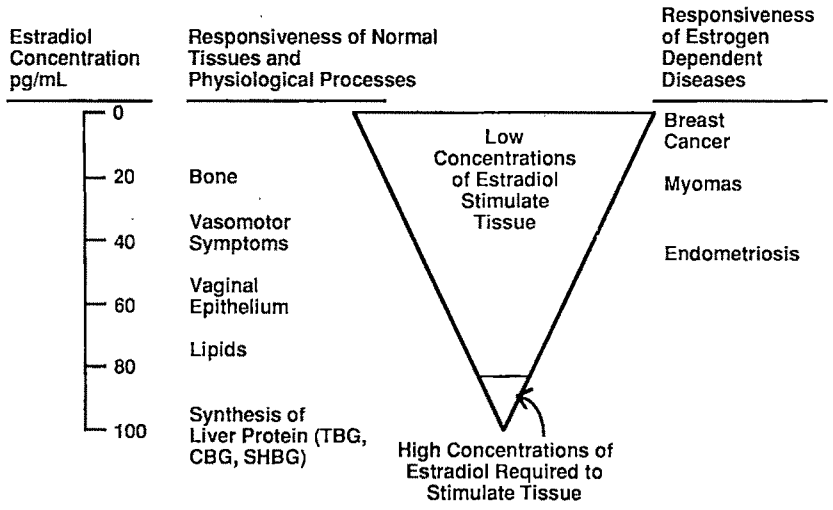


Fig. 1. Hierarchy of tissue and disease response to various concentrations. Calcium metabolism and bone may be more sensitive to estradiol than vaginal epithelium and lipid metabolism. *TGB*, Thyroid-binding globulin; *CBG*, cortisol-binding globulin; *SHBG*, sex hormone–binding globulin.

Tissues vary in their sensitivity to estradiol

The essence of the estrogen threshold hypothesis is that tissues vary in their sensitivity to estradiol and that a concentration of estradiol (30 to 45 pg/ml) that will partially prevent bone loss may not stimulate endometriotic lesions to grow. Evidence to support the concept that tissues vary in their sensitivity to estradiol has been provided by Chetowski et al.[11] In a brilliantly designed and executed study, Chetowski et al. randomized menopausal, hypoestrogenic women to receive one of four different doses of transdermal estradiol: 25, 50, 100, or 200 μg/day or two doses of oral conjugated estrogens, 0.625 or 1.25 mg/day.

Before estradiol replacement therapy was initiated, women had a circulating estradiol concentration of less than 10 pg/ml, elevated urinary calcium excretion, elevated serum gonadotropin concentrations, decreased vaginal superficial epithelial cells, and elevated low-density lipoprotein cholesterol. The transdermal estradiol replacement therapy produced an increase in circulating estradiol proportional to the dose administered. For example, at doses of 25, 50, 100, and 200 μg/day, the circulating estradiol concentrations were approximately 28, 25, 65, and 110 pg/ml, respectively. At the lowest estradiol replacement dose, 25 μg/day, a significant decrease in urinary calcium excretion and follicle-stimulating hormone concentration was noted. However, an estradiol replacement dose of 25 μg/day produced no significant change in luteinizing hormone concentration, vaginal superficial epithelial cells, or circulating lipids. This suggests that calcium turnover may be exquisitively sensitive to estradiol. This contrasts to the relative lack of sensitivity of vaginal epithelium and circulating lipids to small doses of estradiol. At an estradiol replacement dose of 100 μg/day, urinary calcium excretion was suppressed, follicle-stimulating hormone and luteinizing hormone concentrations were decreased, and vaginal superficial cells increased. This indicates that to increase the percentage of vaginal superficial cells, more estradiol is needed than required to suppress urinary calcium excretion.

These observations suggest a hierarchy of responsiveness of various organs to estradiol. Fig. 1 depicts this hierarchy. From the studies of Chetowski et al.,[1] it appears that the hierarchy of most sensitive to least sensitive estrogen-responsive processes is: (1) calcium turnover, (2) gonadotropin secretion, (3) vaginal epithelial growth, (4) lipid production, and (5) liver protein production, such as sex hormone–binding globulin production. In a like manner, estradiol-dependent disease processes also appear to have a hierarchy of responsiveness to estradiol. Breast cancer can be very sensitive to the growth-promoting effects of estradiol concentrations as low as 10 to 20 pg/ml.[12] To reduce myoma volume in premenopausal women by 50%, estradiol concentrations in the range of 15 to 25 pg/ml must be achieved.[13, 14] Estradiol concentrations in the range of 30 pg/ml reliably produce regression in endometriotic lesions.[15] The hierarchy of most sensitive to least sensitive estrogen-responsive disease processes appears to be: (1) breast cancer, (2) myomas, and (3) endometriosis (Fig. 1).

The estrogen threshold hypothesis: Surgical oophorectomy and estrogen add-back

At very low concentrations of estradiol (in the range of 10 to 20 pg/ml), most endometriotic lesions regress, but bone mineral loss can be substantial. At high estradiol concentrations (in the range of 100 to 300 pg/ml), endometriotic lesions can grow, but bone min-

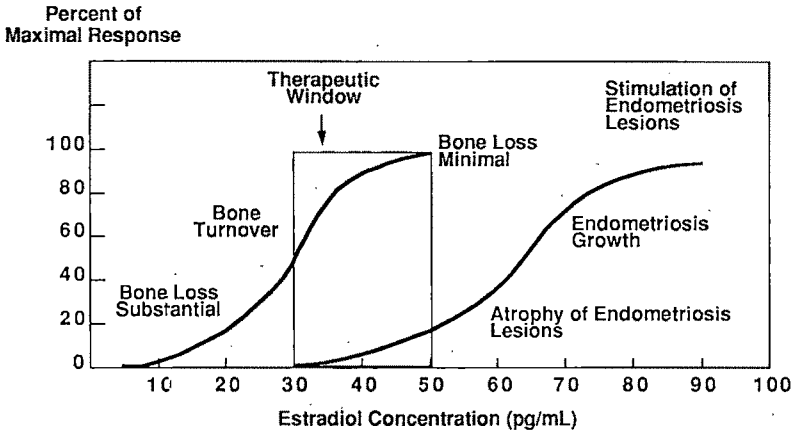


Fig. 2. Estradiol therapeutic window. The concentration of estradiol required to cause growth of endometriosis lesions may be greater than the concentration required to stabilize bone mineral density.

eral density does not decrease. Evidence to support the concept that there may be an "estradiol therapeutic window" comes from studies in which surgical oophorectomy plus estrogen add-back was used to successfully treat women with endometriosis.[16-18] Before reviewing these studies, it should be noted that no randomized prospective studies to assess the risks and benefits of estrogen replacement therapy in women with endometriosis who have undergone bilateral oophorectomy and hysterectomy for the treatment of endometriosis have been reported. All the studies to date are retrospective studies with no control group and are subject to a variety of possible biases. However, the results of all three retrospective studies are similar: estrogen replacement therapy after total hysterectomy and bilateral oophorectomy (TAH-BSO) was associated with a low rate (<10%) of recurrence of endometriosis. For example, Ranney[18] reported on 144 women with endometriosis who were treated with definitive surgery (TAH-BSO). Of these women, 48 patients were not given estrogen replacement therapy because 13 had severe involvement of the bowel or ureters by endometriosis, 15 had endometrial, ovarian, or breast cancer, and 20 had no menopausal symptoms. The remaining 96 women received ethinyl estradiol in oral doses between 0.02 and 0.05 mg daily. For 32 women, the hormone therapy was started more than 3 months after the surgery. In 3 of these 96 patients, the symptoms of endometriosis recurred, which required the discontinuation of the estrogen therapy. Gray[16] observed similar results, with 8 of 56 women developing recurrent endometriosis during treatment with conjugated estrogens (0.3 or 0.6 mg) after TAH-BSO. The observation that doses of estrogen known to protect bone mineral density can be safely prescribed to most women with endometriosis who undergo TAH-BSO strongly suggests the existence of an estrogen therapeutic window (Fig. 2).

If surgical oophorectomy plus estrogen add-back can be successful in the treatment of endometriosis, then medical oophorectomy with the GnRH analogs plus estrogen add-back also might be successful. To date no large randomized prospective trials to test this hypothesis have been published. Such studies are currently being conducted. Until these studies are completed, the precise risks and benefits of a treatment strategy combining long-term GnRH analog therapy with estrogen-progestin add-back will be unclear. However, from my clinical experience in the treatment of women with endometriosis and pelvic pain, the regimens combining long-term GnRH analog therapy with low-dose estrogen replacement appear to be effective in controlling pelvic pain and minimizing the loss of bone mineral density.

The estradiol therapeutic window

The basic tenant of the estradiol threshold hypothesis is that tissues vary in their sensitivity to estradiol (Fig. 1). Bone and calcium metabolism may be very sensitive to estradiol, whereas many endometriotic lesions are somewhat less sensitive to estradiol. This difference in estradiol sensitivity means that an estradiol therapeutic window may exist (Fig. 2). At estradiol concentrations of less than 20 pg/ml, endometriotic lesions will tend to atrophy, but loss of bone mineral density will be substantial. At estradiol concentrations of greater than 100 pg/ml, endometriotic lesions will flourish, but loss of bone mineral will not occur. At estradiol concentrations of 30 to 45 pg/ml, many endometriotic lesions may not be stimulated to grow, and

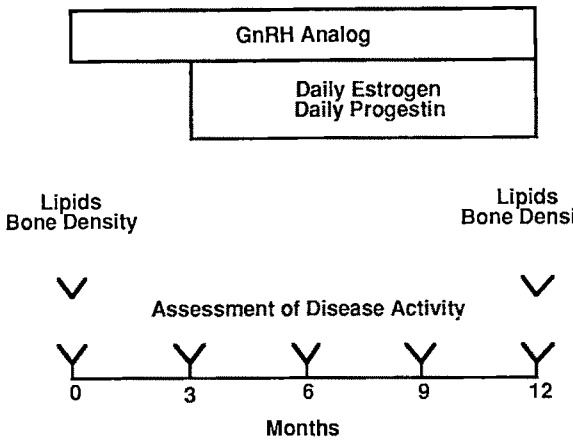


Fig. 3. Regimen I. GnRH analog is used as a single agent for 3 months to produce regression of endometriotic lesions. Combined GnRH analog plus daily estrogen-progestin therapy is then initiated to try to prevent the adverse effects associated with a prolonged hypoestrogenic state.

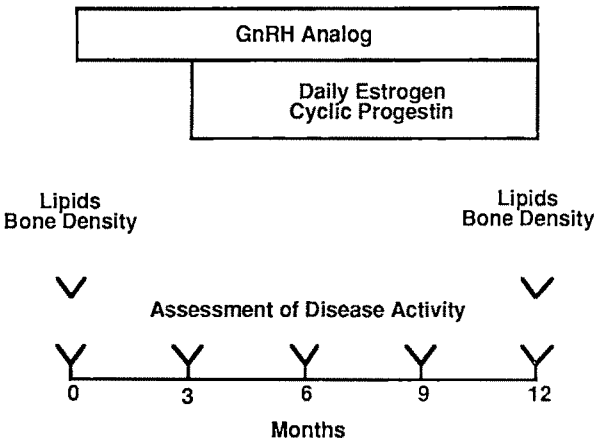


Fig. 4. Regimen II. GnRH analog is used as a single agent for 3 months to produce regression of endometriotic lesions. Combined GnRH analog plus daily estrogen and cyclic progestin therapy is then initiated to try to prevent the adverse effects associated with a prolonged hypoestrogenic state.

bone loss may be minimal. Whether an estradiol therapeutic window exists in every woman is unclear. An occasional endometriotic lesion may be extremely sensitive to the stimulatory effects of estradiol. In addition, the "width" of the window may vary from woman to woman. In some women, the window may be extremely narrow, and it may be difficult to target the circulating estradiol concentration to stay within the estradiol therapeutic window.

The estrogen threshold hypothesis: Practical applications in the treatment of endometriosis

The design of clinical trials to evaluate the efficacy of medical treatment of endometriosis is complicated by the need for repetitive laparoscopies to objectively evaluate the extent of disease. Many women are unwilling to enter study protocols in which two or more laparoscopies will be required. Consequently, many investigators interested in the biology and treatment of estrogen-dependent gynecologic diseases have turned to uterine leiomyomas as their experimental model of choice. The prevalence of uterine leiomyomas is very high; thus many women are eligible for study protocols. In addition, the objective response of uterine myomas to medical treatment can be monitored with noninvasive techniques such as sonography, magnetic resonance imaging, and physical examination. Many of the management principles generated in studies of the medical treatment of myomas may be applicable to the treatment of endometriosis. A basic management principle in the use of long-term combined GnRH analog plus estrogen-progestin add-back therapy is to treat with the GnRH analog alone for 3 months and then

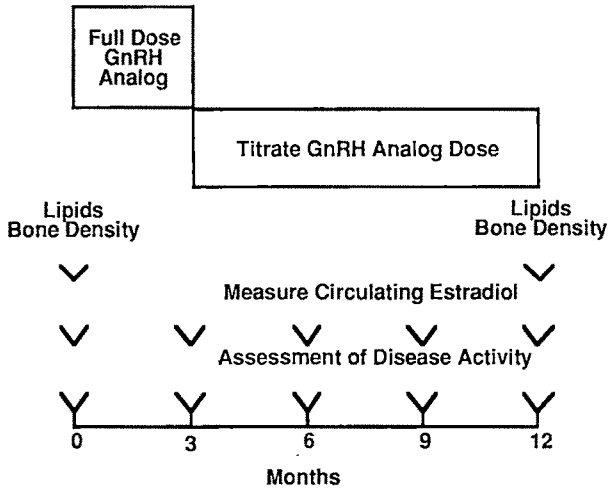


Fig. 5. Regimen III. GnRH analog is used as a single agent at full dose for 3 months to produce regression of endometriotic lesions. The dose of GnRH analog is then titrated in an attempt to produce a circulating estradiol in the range of 30 to 45 pg/ml.

"add-back" estrogen plus progestin.[13, 19, 20] This strategy allows the GnRH analog to produce near-maximal regression of disease before steroid add-back. It is possible that the hypoestrogenic state forces the estrogen-dependent disease into a state of "hibernation" in which it is less responsive to the possible stimulating effects of the estrogen-progestin add-back. This basic principle is incorporated into all the treatment regimens described below.

Four regimens that I have used in the long-term hor-

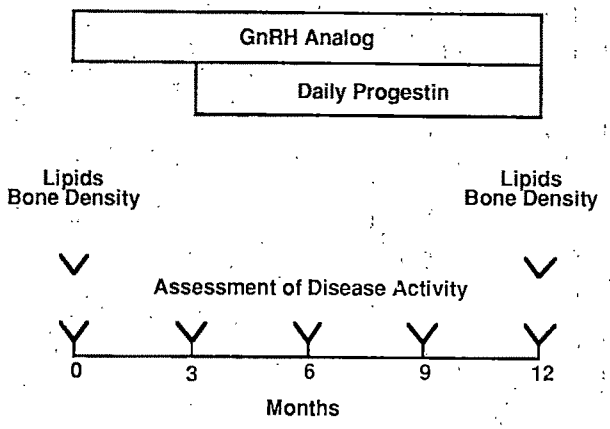


Fig. 6. Regimen IV. GnRH analog is used as a single agent for 3 months to produce regression of endometriotic lesions. Combined GnRH analog plus daily progestin therapy is then initiated to try to prevent the adverse effects of a hypoestrogenic state.

monal treatment of endometriosis are presented in Figs. 3 to 6. The efficacy of these regimens has not been documented in large prospective, randomized trials. Surgical documentation of endometriosis is necessary for patients to be entered into these treatment regimens. For all long-term regimens (>6 months of GnRH analog therapy), measurement of serum lipids and bone mineral density may be useful to gauge potential adverse effects of these protocols. The clinician might also want to ensure that the patient is receiving adequate dietary calcium.

Regimen I involves the use of a GnRH analog alone for 3 months followed by the combined use of GnRH analog plus continuous daily estrogen plus progestin. In initiating the estrogen add-back, low doses of estrogen can be used (0.3 mg of conjugated estrogen daily; 50 μg/day of transdermal estradiol), and the dose of estrogen can be increased (0.625 mg of conjugated estrogen) if symptoms and physical findings suggest that the endometriosis is in good control. Oral medroxyprogesterone, 2.5 mg daily, or norethindrone, 0.35 mg daily, can be used as the progestin.

In regimen II, a GnRH analog alone is used for 3 months followed by the combined use of GnRH analog plus continuous daily estrogen and cyclic progestin. As in regimen I, the clinician may want to use low doses of estrogen in initiating the estrogen add-back (0.3 mg of conjugated estrogen daily) and the estrogen dose can be increased (0.625 mg of conjugated estrogen daily) if symptoms and physical findings suggest that the endometriosis is in good control. In women receiving the combined GnRH analog plus daily estrogen replacement therapy, the frequency of progestin cycles needed to prevent endometrial hyperplasia remains to be determined. It is conceivable that endometrial hyperplasia could be prevented by the use of a progestin cycle every other month rather than every month. A disadvantage to regimen II is that cyclic menses, at best with minimal bleeding, may occur. An advantage of regimen II is that minimal doses of progestin are used, which reduces the adverse effects of a progestin on circulating lipids.

In regimen III, a novel approach to "estrogen add-back" is used: adjust the dose of GnRH analog to allow the ovary to produce enough estradiol to achieve a circulating estradiol concentration in the range of 30 to 45 pg/ml. In regimen III, full-dose GnRH analog treatment (e.g., nafarelin nasal solution, 200 μg twice daily) is used for 3 months. The dose of the GnRH analog is then adjusted to produce the desired circulating estradiol concentration. Previous studies suggest that a dose-response relationship exists between nafarelin and circulating estradiol.[15, 21] In using such a regimen, care must be taken to avoid missed GnRH agonist doses. In regimen III the possibility exists that the pituitary-ovarian axis could escape from GnRH analog suppression, and a dominant follicle and ovulation could occur. Patients treated with GnRH analogs should use non-hormonal contraceptives to avoid the rare possibility of a unintended pregnancy.

Regimen IV involves the use of a GnRH analog alone for 3 months followed by the combined use of a GnRH analog plus a daily progestin. Judd et al. have evaluated the use of regimens of combined GnRH analogs plus daily progestin in the treatment of endometriosis.[22, 23] The progestin appears to suppress vasomotor symptoms caused by the GnRH analog. However, progestins are probably not as effective as estrogen in protecting bone mineral density. In addition, high-dose progestins may suppress circulating concentrations of high-density lipoprotein cholesterol, which results in an atherogenic lipid profile. If an estradiol therapeutic window exists, it is probably better to use estrogen add-back than progestin only add-back in combined GnRH analog plus steroid add-back regimens.

Conclusions

Estradiol is an important growth factor for endometriotic lesions.

Hypoestrogenic states clearly result in the atrophy of endometriosis lesions.[7-10]

A major question that is still unresolved is: What *precise* concentration of estradiol is required to produce atrophy of endometriotic lesions? Data that definitively answer this question will allow us to develop truly scientific regimens for the treatment of endometriosis.

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Treatment of uterine fibroids: Current findings with gonadotropin-releasing hormone agonists

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The gonadotropin-releasing hormone agonists have potential benefit as presurgical adjuncts in the management of uterine leiomyomas or fibroids. Uterine fibroids contain estrogen receptors and are responsive to therapeutic hormonal manipulation; gonadotropin-releasing hormone agonists are effective by inducing a state of hypoestrogenism. Clinical trials with gonadotropin-releasing hormone agonists consistently have demonstrated efficacy for decreasing both myoma size and uterine volume. The advantages of the preoperative use of gonadotropin-releasing hormone agonists include a reduction in uterine and myoma size and vascularity and potentially improved operative technique and uterine cavity integrity. Ongoing clinical trials will be needed to confirm the role of gonadotropin-releasing hormone agonists in the treatment of uterine fibroids. (AM J OBSTET GYNECOL 1992;166:746-51.)

Key words: Gonadotropin-releasing hormone analog, leiomyoma, uterine fibroids, leuprolide, nafarelin

Uterine fibroids is a common medical problem among women, and surgery is often recommended for management. Recently, gonadotropin-releasing hormone (GnRH) agonists have been investigated for use in uterine fibroid disease, but many questions remain about their role in overall management. To help clarify the role of GnRH agonists in myomatous uteri, this article will review the effect of GnRH agonists on uterine myomas and the uterus. The clinical implications of these actions and the potential disadvantages of GnRH agonists will also be discussed. Finally, a brief summary of the current role of GnRH agonists in the management of uterine myomas will be offered.

Uterine fibroids

Leiomyomas or uterine fibroids are neoplasms of the smooth muscle found in about 20% of women over the age of 30 years.[1] Although uterine leiomyomas are the most common solid tumor of the female genital tract, they are benign. Thus therapeutic intervention is not required for all women with uterine fibroids. Indications for the treatment of uterine myomas include menorrhagia with iron deficiency anemia, uterine pressure or pain, rapidly increasing fibroid size, recurrent abortion with endometrial cavity distortion, and infertility with endometrial cavity distortion. Unexplained or refractory infertility may also be an indication for myomectomy.[2]

Until recently, there has been no generally accepted medical therapy for the management of leiomyomas. Rather, management of patients with symptoms of uterine myomas has traditionally been surgical ranging from myomectomy to hysterectomy. Of the 650,000 hysterectomies performed annually in the United States, approximately 27% or 175,000 are performed for uterine myomas.[3] Myomectomy is performed in about 18,000 patients per year. Myomectomy has a recurrence rate of 15% (range, 4% to 59%) and a repeat operation rate of 10% (range, 3% to 21%), and subsequent hysterectomy is required in an additional 1% to 5% of patients.[4, 5]

Although the pathogenesis of myomas is not well established, it has been recognized that fibroid growth and maintenance are stimulated by estrogen and are affected by hormonal cyclic changes.[6] Estrogen and progesterone receptors have been identified in myomatous tissue.[7] Because leiomyomas can be influenced by changes at the estrogen and progesterone receptors, GnRH agonists have been investigated as a potential treatment.

Several pilot and small studies have been reported in which GnRH agonists were evaluated for the treatment of uterine fibroids.[1, 2, 8-11] Potential benefits of GnRH agonist therapy are a reduction in uterine fibroid size and uterine volume, decreased preoperative and perioperative blood loss, and an improvement in operative conditions.

Effect of GnRH agonists on fibroid size

A primary effect of GnRH agonists on uterine fibroids should be to decrease fibroid size. In one study, the effect of daily administration of nafarelin on uterine and myoma size was evaluated in 10 patients with the use of magnetic resonance imaging.[12] After 6

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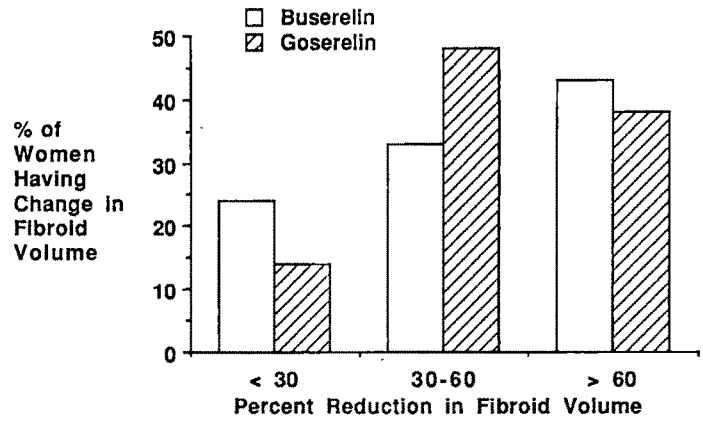


Fig. 1. Proportion of women having a change in fibroid volume from baseline to 6 months after treatment with buserelin (n = 21) or goserelin (n = 21). (Modified from Costantini et al. Eur J Obstet Gynecol Reprod Biol 1990;37:63-9, by permission of Elsevier Science Publishers.)

months of treatment, the size of the largest myoma decreased by a mean of 46% from baseline in 9 of 10 women. Uterine volume decreased by an average of 57% in all treated patients. Of note, both small and large myomas responded to nafarelin therapy, but consistent with other trials, myomas became enlarged again after discontinuation of nafarelin in several women.

In a more recent trial, Costantini et al.[13] compared the effects of 6 months of treatment with intranasal buserelin or subcutaneous goserelin on myoma size. A reduction of more than 30% in myoma volume was seen in 34 of 42 (81%) patients, and no difference was seen between the two GnRH agonists (Fig. 1). As reported in other clinical trials, regrowth of the myomas followed the discontinuation of therapy.[14]

One of the largest trials conducted to date with a GnRH agonist was a multicenter, randomized, placebo-controlled trial in which the effects of intranasal nafarelin on menstrual blood loss and uterine and fibroid volume were evaluated in 166 patients scheduled for elective hysterectomy (Ylikorkala, personal communication). Patients scheduled for hysterectomy for nonmalignant conditions, including uterine fibroids, menorrhagia, or pelvic pain, were evaluated by ultrasound examination. Patients were randomized in a 2:1 ratio to treatment with either intranasal nafarelin, 200 μg twice daily, or placebo for 3 months or until hysterectomy. In patients with fibroids, the total volume of the three largest fibroids decreased by 31% from admission to 3 months in the nafarelin group but increased by 1.2% in the placebo group (p = 0.024; Fig. 2). Assessment of the largest fibroid at the time of surgery showed a decrease in the volume of the largest fibroid with nafarelin compared with placebo (p = 0.055).

All the studies done to date with GnRH agonists show a variable but significant decrease in myoma size; however, in most instances, myomas returned to pretreatment size after discontinuation of GnRH agonist therapy. The reasons for this response within a few months to GnRH agonist therapy have been studied. Pasqualini et al.[15] looked at the effect of a GnRH agonist, decapeptyl, on hormone levels and receptors in uterine myomas and found that myoma tissue was very sensitive to changes in hormone levels. This occurs because myoma tissue has receptors for estrogen and progesterone and thus is affected by both cyclic changes in hormone levels and pharmacologic therapy such as GnRH agonists, which alter hormonal levels.[16] The number of estrogen receptors decreases during the postovulatory phase and is greatest during the follicular phase. Further, estrogen and progesterone receptors are more numerous in submucous than subserosal myomas. This has led to the hypothesis that submucous myomas may respond better to GnRH agonist therapy than large subserosal myomas. In addition, the effect of GnRH agonists on myomas is to decrease cell size and intracellular matrix but without changes in the number of cells. Therefore it is not surprising that after the GnRH agonist is discontinued, myomas return to pretreatment size.

Effect of GnRH agonists on uterine size

GnRH agonists are also known to decrease uterine size. In a prospective randomized trial, Friedman et al.[14] demonstrated a reduction in uterine volume with leuprolide acetate. Eighteen women were randomly assigned to receive monthly intramuscular injections of leuprolide or placebo for 24 weeks. A significant ($p < 0.05$) reduction in uterine volume was noted at 12 and at 24 weeks in the leuprolide group compared with pretreatment volume (Fig. 3). However, uterine volume returned to 88% of pretreatment levels by 3 months after discontinuation of GnRH therapy.

In another large double-blind multicenter trial, leu-

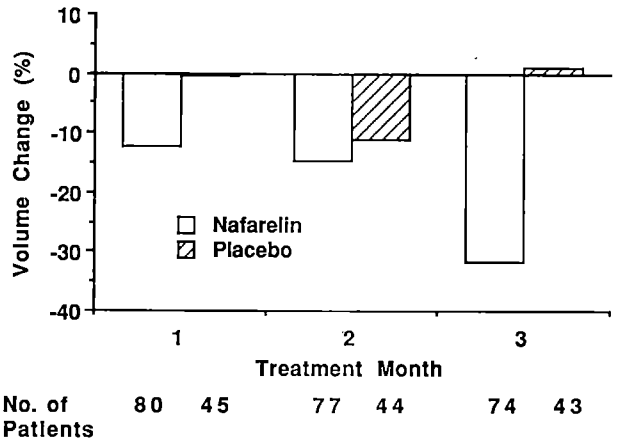


Fig. 2. Median percentage change in volume of the largest three fibroids (Ylikorkala, personal communication). $p = 0.024$ for nafarelin vs placebo by two-way analysis of variance.

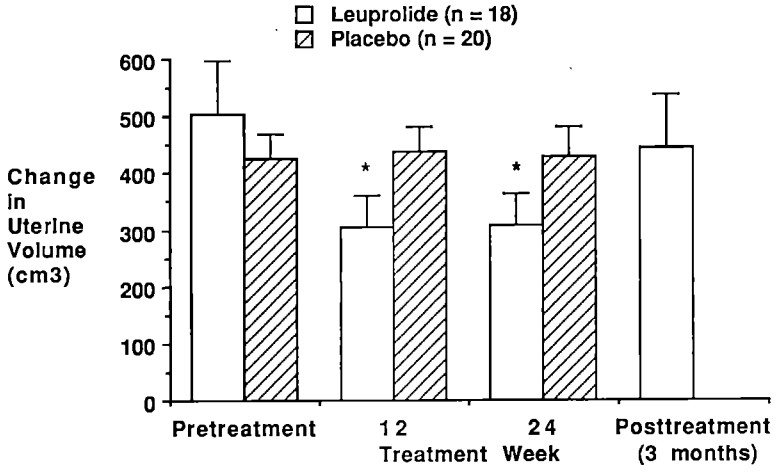


Fig. 3. Change in uterine volume (mean ± standard error) during treatment with depot leuprolide acetate or placebo in women with uterine fibroids.[14] *$p < 0.05$ vs pretreatment by the Dunnett t test. (From Friedman AJ, Harrison-Atlas D, Barbieri RL, Benacerraf B, Gleason R, Schiff I. A randomized, placebo-controlled, double-blind study evaluating the efficacy of leuprolide acetate depot in the treatment of uterine leiomyomata. Fertil Steril 1989;51:251-6. Reproduced with permission of the publisher, The American Fertility Society.)

prolide acetate or placebo were administered to 128 women with leiomyomata uteri every 4 weeks for 24 weeks.[17] Mean uterine volume decreased by 45% in patients treated with leuprolide compared with a 5% increase in those given placebo. Uterine volume returned to pretreatment size by 24 weeks after leuprolide was stopped.

The Finnish study that used nafarelin also showed that both the length and volume of the myomatous uteri were decreased significantly ($p < 0.05$) in the nafarelin group compared with the placebo group (Ylikorkala, personal communication). These findings were confirmed during surgery where the uterine weight was measured. There was a significant decrease in uterine weight in the nafarelin group compared with the placebo group in all patients and in the patients with fibroids only. Thus the results from these studies demonstrate that GnRH agonists reduce uterine and myoma size.

Implications of GnRH treatment of uterine fibroids

With a documented reduction in uterine and myoma size, the question becomes "What are the implications of the effects of GnRH agonists in the clinical management of myomas?" One of the potential benefits of preoperative GnRH agonist treatment of uterine fibroids is a reduction in menstrual blood loss. Results

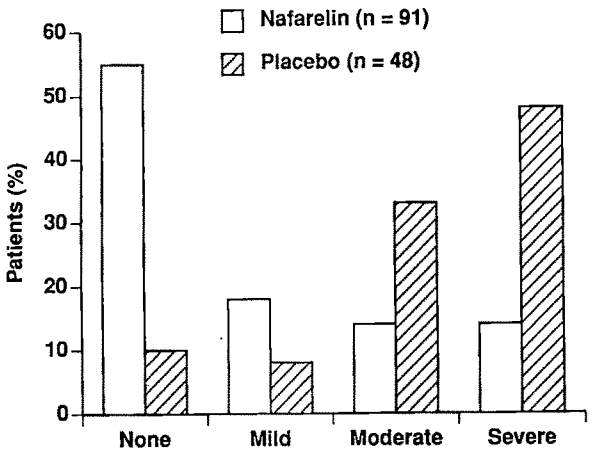


Fig. 4. Severity of menstrual blood loss at 3 months in women treated with nafarelin or placebo before elective hysterectomy (Ylikorkala, personal communication). $p < 0.001$ for nafarelin vs placebo by Cochran-Mantel-Haenszel test.

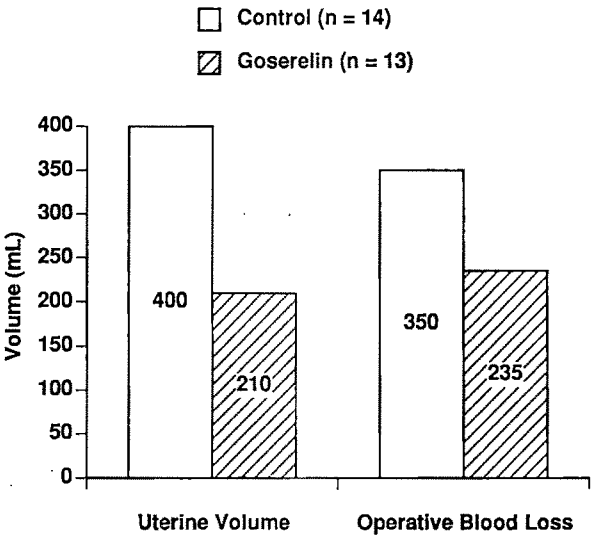


Fig. 5. Uterine volume and operative blood loss in women with symptomatic uterine fibroids treated for 3 months before elective hysterectomy. $p < 0.01$ for goserelin vs placebo by Wilcoxon two-sample test. (Modified from Lumsden MA et al. Lancet 1987;1:36-7.)

from the Finnish study confirm this effect (Ylikorkala, personal communication). In patients with severe blood loss at baseline, 55% of those randomly assigned to nafarelin therapy had no bleeding at the end of the 3-month treatment period compared with 10% of the placebo-treated patients ($p < 0.001$). In contrast, 48% of patients receiving placebo continued to have severe menstrual bleeding at the end of therapy compared with only 14% of patients receiving nafarelin (Fig. 4). In addition, a mean increase of 4.2% in hemoglobin levels was noted before surgery in patients treated with nafarelin compared with a decrease of 2.7% in the placebo group ($p = 0.032$).

In another study, patients were treated with 3.6 mg of subcutaneous goserelin every 28 days for 6 months.[10] In addition to a reduction in myoma and uterine size, there was a reduction in both menstrual blood loss and blood loss at the time of surgery (Fig. 5). Similar findings were reported in the large leuprolide study.[17] In addition to improvement in symptoms in most patients, 37 of 38 patients (97%) with menorrhagia at baseline had resolution by the end of treatment with leuprolide.

The decrease in blood loss and the increase in hemoglobin and hematocrit afforded by GnRH agonist therapy have several potential benefits, although it must be emphasized that prospective randomized studies proving these potential benefits have not yet been performed. The patient's hematocrit level may be sufficient to allow the patient to give autologous blood donations before surgery. Further, the lessened perioperative blood loss may reduce the need for blood transfusion and hysterectomy at the time of surgery. Thus the risks associated with blood transfusion, which are often overlooked or underestimated, may be reduced. Between 8% and 18% of patients require blood or blood products at surgery; the current risk of hepatitis is approximately 8% per transfusion.[5, 18, 19] Other potential advantages from the use of GnRH agonists may result from a reduction of uterine myoma size. These include the use of more conservative surgery; that is, the condition may be managed with myomectomy rather than hysterectomy. Smaller and fewer incisions may result in decreased tissue trauma and increased preservation of normal tissue, possibly reducing adhesion formation after surgery. Other potential advantages are listed in Table I.

Table I. Potential advantages of a reduction in uterine and myoma size by GnRH agonists

Reduced vascularity
• Reduced menorrhagia
• Increased hematocrit and iron
• Autologous donation
• Less operative blood loss
• Reduced risk of blood transfusion
• Reduced risk of hepatitis
Operative technique
• Hysterectomy less likely
• Operative laparoscopy more likely
• Fewer, smaller incisions
• Less tissue trauma
• Less risk of injury
• Pfannenstiel incision more feasible
• Vaginal hysterectomy more feasible
Integrity of uterine cavity
• Hysteroscopic approach
• Avoidance of uterine cavity at laparotomy

Disadvantages of GnRH agonist therapy

Treatment with GnRH agonists is not benign. The known hypoestrogenic side effects are hot flashes and bone loss. About 2% of patients receiving GnRH agonist therapy can experience a very profuse vaginal hemorrhage requiring immediate surgical management.[20] This serious clinical problem has been reported to date only in patients with submucous myomas. The cause of this adverse event is thought to be a reduction in myoma size and a loss of mechanical compression of the vessels within the uterus. Also, painful fibroid degeneration may occur. There is also concern about making the fibroid so small as to avoid detection at the time of surgery.

Some investigators have been concerned that myomas may be reduced to an undetectable size by preoperative GnRH agonist use. Results from a recent trial of buserelin before myomectomy suggested that this phenomenon occurs.[21] In this randomized trial, a 44% decrease in uterine volume was observed during buserelin therapy in eight patients compared with no change in 16 untreated control patients. Intraoperative blood loss and postoperative morbidity did not differ between groups. However, at the 6-month postoperative evaluation, only 2 of 16 (13%) patients in the untreated group had myomas smaller than 1.5 cm in size compared with 5 of 8 (63%) patients in the buserelin group. These data suggest that small myomas may have been missed at the time of surgery because of preoperative treatment with buserelin. The results of this study need to be confirmed by others, and if true, its clinical relevance established.

Strategies for managing uterine myomas

Despite the promising results of studies with GnRH agonists for the treatment of uterine fibroids, surgery remains the treatment of choice. Several different procedures are appropriate including myomectomy by either laparoscopy, laparotomy, or hysteroscopy or hysterectomy by various surgical approaches. However, the use of a GnRH agonist as a preoperative adjunct has some potential benefits to the patient. Preoperative use of GnRH agonists may increase the feasibility of using myomectomy instead of hysterectomy, increase the use of laparoscopic, hysteroscopic, or vaginal procedures, preserve normal uterine tissue, facilitate the operative technique, and reduce blood loss. The choice of therapy will depend on the surgical aspects of treatment, but also on the size and number of myomata, the age of the patient, and the desire to preserve fertility and uterine function.

One innovative strategy for managing uterine fibroids with medical therapy only is to combine the GnRH agonist with an estrogen-progestin combination. In a preliminary trial, five women with leiomyomata uteri were treated with leuprolide for 24 weeks.[22] After 3 months, conjugated estrogens were added on days 1 to 25 and medroxyprogesterone acetate from days 16 to 25. Patients were followed for as long as 2 years. A 49% decrease in uterine volume was seen at 3 months, but no further decrease was seen during the next 2 years. There was also a decrease in vasomotor symptoms, and bone density was preserved. These results were encouraging, but additional data obtained on more patients and a longer duration of study have shown a regrowth of the myomas in some patients taking this regimen. This confirms not only that leiomyomata and myometrium are estrogen sensitive, but suggests that an estrogenic threshold exists for uterine growth. It can be seen that different treatment strategies that use GnRH agonists show promise, but the optimal approach to medical treatment of uterine fibroids is unclear. Further studies, many currently ongoing, will help us understand and use GnRH agonists better in the future.

In conclusion, results from clinical trials have consistently shown the efficacy of GnRH agonists as a preoperative adjunct for reducing myoma and uterine size and preoperative and perioperative blood loss. Uterine myomas are very sensitive to hormonal changes probably mediated through estrogen and progesterone receptors, the likely mechanism by which GnRH agonists can effect changes. Although the role of the GnRH agonists in the management of uterine leiomyoma has not been fully defined, use as a presurgical adjunct may allow preservation of uterine tissue because of removal of smaller myomas and more conservative surgical procedures.

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Gonadotropin-releasing hormone agonists: Strategies for managing the hypoestrogenic effects of therapy

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Gonadotropin-releasing hormone agonists have been shown to be comparable with danazol for the treatment of endometriosis. These results are important because danazol is associated with a significant number of side effects, particularly androgenic effects, such as weight gain and acne. Although gonadotropin-releasing hormone agonists are associated with hypoestrogenic side effects, such as hot flashes and reversible bone density loss, recent studies suggest that the risk of these side effects may be modified when gonadotropin-releasing hormone agonists are combined with a progestin. These findings suggest that such regimens may further enhance the clinical usefulness of gonadotropin-releasing hormone agonist therapy for endometriosis and other estrogen-mediated diseases. (Am J Obstet Gynecol 1992;166:752-6.)

Key words: Hypoestrogenism, norethindrone, vasomotor symptoms

Gonadotropin-releasing hormone (GnRH) agonists were discovered in the early 1970s and subsequently have been studied extensively for the treatment of a broad range of endocrine disorders, including endometriosis and uterine leiomyomata.[1] Two GnRH agonists, nafarelin and leuprolide, have been approved for the treatment of endometriosis, and clinical investigations with these and other GnRH agonists are ongoing for other indications.

The GnRH agonists were first used clinically in the 1980s. Soon after it was realized that although these potent agents produce important pharmacologic effects, they also were associated with some side effects.[2] GnRH agonists suppress luteinizing-hormone and follicle-stimulating hormone to turn off gonadal production of estradiol and testosterone.[1] Thus the side effects associated with GnRH agonists appear to be linked to the rapid and complete suppression of circulating estrogen induced by GnRH agonists. Prominent among these are typical hypoestrogenic side effects such as hot flashes and vaginal dryness.[3] Of more concern are reductions in bone density. Although some investigators have found no significant loss in bone density,[4] others have found reversible bone density loss,[5] and still others have found the loss to be irreversible.[6] Nevertheless, in many individuals these adverse effects are not considered an obstacle to routine use of GnRH agonists because the overall benefits of treatment are favorable. Moreover, the desire to modify these risks by combining GnRH agonists with other agents has prompted a series of experimental and clinical studies to identify treatment strategies that will ameliorate these side effects.

Of the potential approaches to this situation, two strategies are most promising. One is to first suppress circulating estrogen with a GnRH agonist, thereby inducing control of endometriosis, and then add-back exogenous estrogen in small amounts to reverse some of the unwanted hypoestrogenic effects. The other approach is to administer small amounts of exogenous progestin concurrently with the GnRH agonist in an attempt to circumvent the hypoestrogenic effects altogether.

Pilot studies of add-back regimens of estrogen have yielded promising results, but clinical trials with this approach have been initiated only recently. More widely evaluated has been the concurrent administration of progestins, which has now been studied in several trials. Although these studies have yet to identify a regimen that eliminates side effects while preserving the efficacy of the GnRH agonist, they have demonstrated that this approach is viable and have prompted a more vigorous search for an optimal combination. At my institution, where some of the initial studies in this area were performed, controlled trials have suggested that it is possible to deliver effective but more tolerable doses of GnRH agonists when progestins are used to modify associated adverse effects.

GnRH agonist plus medroxyprogesterone acetate

A pilot study was performed at my institution with the use of medroxyprogesterone acetate in an attempt to modify GnRH agonist side effects.[7] Eight women with endometriosis were studied. Each received a daily subcutaneous dose of a GnRH agonist plus medroxy-

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progesterone acetate, 20 to 30 mg daily for 24 weeks. Patients were evaluated at baseline and at 6 months with laparoscopy and bone density measurements. Objective and subjective hot flash scores were obtained routinely. Results with this combination were compared with results obtained previously in patients treated with a GnRH agonist alone.[8]

Serum estrogen and gonadotropic hormone levels were reduced at 1 month to levels typically seen with a GnRH agonist alone and remained suppressed throughout the course of therapy. Subjective hot flash scores were increased with the combination therapy, but the scores with the GnRH agonist alone were significantly higher during the 6 months of therapy. In addition, objective hot flashes occurred more frequently in patients receiving a GnRH agonist alone than with GnRH agonist plus medroxyprogesterone acetate. In contrast with the effects of a GnRH agonist alone, there was no change in bone density of the distal radius or lumbar spine during treatment with a GnRH agonist plus medroxyprogesterone acetate (Table I).

However, even though ovarian estrogen secretion was reduced to levels seen in castrated women, the regimen failed to significantly reduce symptoms such as pelvic pain. Furthermore, there was no significant improvement in the American Fertility Society score from baseline to 6 months in contrast to a 50% to 60% improvement with the GnRH agonist alone (Fig. 1). Although these results were not expected, they did provide evidence that at least some of the hypoestrogenic effects of a GnRH agonist could be modified with the addition of a progestin. Barbieri et al.[9] found similar results in patients with uterine fibroids. Little or no suppression of fibroid size was observed with the combination of GnRH agonist and medroxyprogesterone acetate.

GnRH agonist plus norethindrone

The reason for the lack of efficacy with a GnRH agonist and MPA is not clear, but it was hypothesized that another progestin with less estrogenic activity may be more effective for reducing hypoestrogenic side effects while maintaining the endometriotic efficacy of the GnRH agonist. In a subsequent study, the GnRH agonist, histrelin, was administered in combination with the 19-nortestosterone derivative, norethindrone.[10] In this study, 10 women with endometriosis were given daily subcutaneous injections of histrelin along with a daily dose of norethindrone that was titrated upward from 0.35 mg to a dose that eliminated vasomotor symptoms. All patients had failed to tolerate or respond to conventional treatment of endometriosis, including danazol, oral contraceptives, conservative surgery, or progestins. Therapy was continued for 24 weeks, and all patients kept diaries beginning 4 weeks before therapy and continuing for 8 weeks after therapy was discontinued. Pain, hot flashes, and additional side effects were recorded and scored in these diaries. Patients also underwent diagnostic laparoscopy before treatment and 4 weeks after the completion of therapy. Bone mineral density was measured before therapy, immediately after therapy, and 24 weeks after treatment. Serum cholesterol and gonadotropin assays were obtained periodically during treatment. The results were compared with 16 patients treated with GnRH agonist alone.[8]

The mean dose of norethindrone was 2 mg during the last 8 weeks of study. A significant reduction in

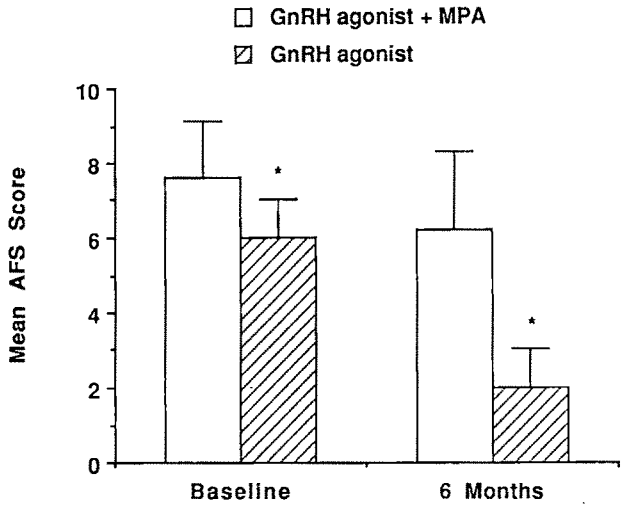


Fig. 1. American Fertility scores (mean ± SE) at baseline and 6 months for patients treated with GnRH agonist plus medroxyprogesterone acetate *(MPA)* (n = 7) or GnRH agonist alone (n = 16). Scores were modified to exclude adhesions not expected to respond to treatment. *$p < 0.001$ for baseline vs 6 months. (From Cedars MI, Lu JKH, Meldrum DR, Judd HL. Obstet Gynecol 1990;75:641-5. Reprinted with permission from The American College of Obstetricians and Gynecologists.)

Table I. Urinary calcium and hydroxyproline excretion and bone density during treatment with GnRH agonist combined with medroxyprogesterone acetate in seven patients with endometriosis

	Duration of therapy	
	Baseline	*6 Mo*
Calcium/creatinine	0.11 ± 0.03	0.14 ± 0.03
Hydroxyproline/creatinine	0.02 ± 0.004	0.03 ± 0.01
Distal radius (gm/cm) SPA	0.93 ± 0.05	0.92 ± 0.06
Lumbar spine (mg/ml) CT	163 ± 7	163 ± 3

From Cedars MI, Lu JKH, Meldrum DR, Judd HL. Obstet Gynecol 1990;75:641-5. Reprinted with permission from The American College of Obstetricians and Gynecologists.

SPA, Single-photon absorptiometry; *CT*, computed tomography.

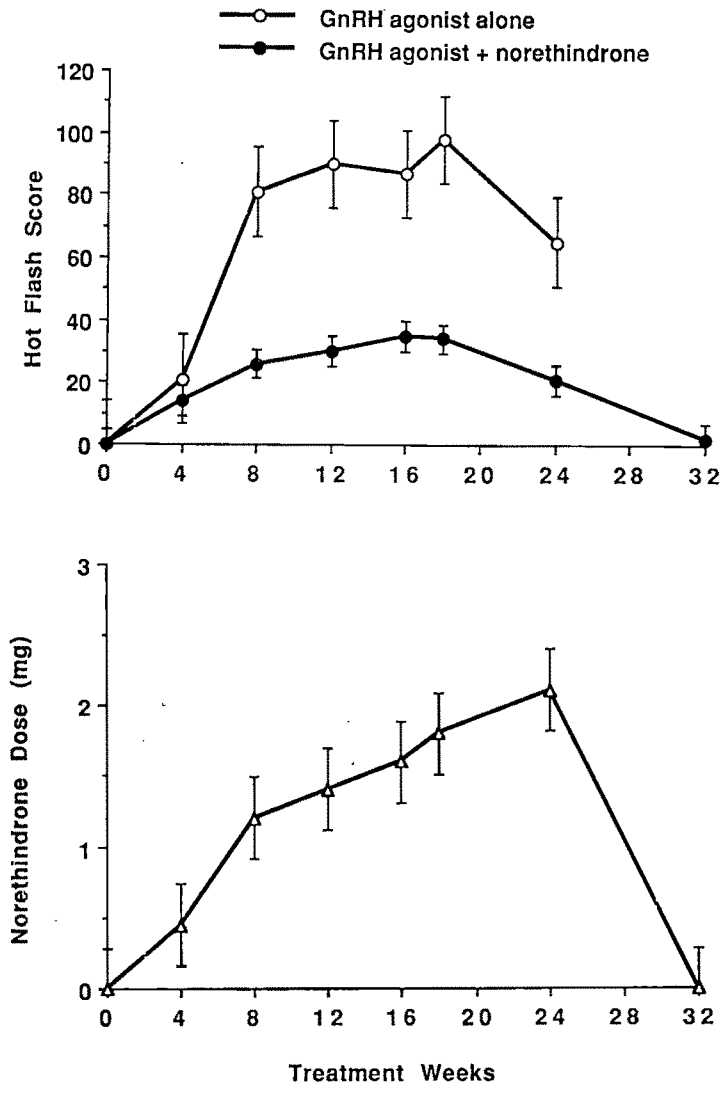


Fig. 2. Subjective hot flash scores **(A)** and mean daily norethindrone dose **(B)** in patients treated with GnRH agonist plus norethindrone *(NE)* or GnRH agonist alone. Values are mean ± SE. (From Surrey ES, Gambone JC, Lu JKH, Judd HL. The effects of combining norethindrone with a gonadotropin agonist in the treatment of symptomatic endometriosis. Fertil Steril 1990;53:620-6. Reproduced with permission of the publisher, The American Fertility Society.)

American Fertility Society scores of endometriosis was observed in patients treated with the GnRH agonist plus norethindrone. The mean American Fertility Society score before therapy was 14.6 ± 3.6 and decreased to 6.4 ± 2.0 after therapy ($p < 0.005$). These results were similar to those observed in women treated with a GnRH agonist alone. More important, the reduction in endometriosis score was accompanied by a significant improvement in pain scores. Pain scores were significantly ($p < 0.005$) reduced at 4 weeks, and this effect was sustained throughout therapy. Again, this pattern of response was similar to results achieved with GnRH agonist alone. Thus the efficacy of the GnRH agonist did not appear to be adversely affected by the addition of this progestin.

Interestingly, the combination regimen was associated with a lower incidence of side effects compared with the control group given the GnRH agonist alone. Subjective hot flash scores were reduced with the GnRH agonist plus norethindrone, and the incidence did not increase beyond week 4 (Fig. 2). Objective hot flashes were seen in only 40% of patients at week 4 and 20% of patients at week 20 with the combination. In the control group receiving the GnRH agonist alone, 60% reported hot flashes at week 4, and 80% reported vasomotor symptoms at week 20. The reduction in vasomotor symptoms in the combination regimen group was associated with increasing dosages of norethindrone. Furthermore, the decrease in vasomotor symptoms with GnRH agonist plus norethindrone occurred

despite a reduction in serum estradiol concentrations to levels expected in the castrated woman.

Bone density of the distal third of the radius was not reduced significantly by the GnRH agonist and norethindrone when measured by single-photon absorptiometry. However, bone density of the lumbar spine declined by a mean of 5.6% at 24 weeks using quantitative computerized tomography (Fig. 3). The change appeared to be completely reversed by 24 weeks after discontinuation of therapy, and no significant difference in bone density of the lumbar spine was detectable between baseline and 24 weeks after therapy. Overall, the findings suggest that the amount of bone loss occurring with GnRH agonist therapy may be either reversible or substantially reduced with the addition of norethindrone.

It is also important that the results from this study on bone density changes were supported further by a report from Riis et al.[11] In this study 17 premenopausal women with endometriosis were treated with nafarelin, 400 μg/day, plus norethisterone, 1.2 mg daily for 6 months, and then norethisterone alone for 6 months. The results were compared with two groups of patients; 20 healthy premenopausal women and 9 premenopausal women with endometriosis treated with nafarelin, 400 μg/day alone. Bone density measurements were obtained in at baseline, 6 months, and 6 months after nafarelin therapy was stopped.

In the group treated with nafarelin only, bone density in the distal radius and lumbar spine decreased by 2% to 6% after 6 months of treatment ($p < 0.05$ vs baseline). All values were not significantly different from baseline at the 6-month posttreatment assessment. In contrast, the group treated with nafarelin plus norethisterone had no significant decrease in lumbar spine density compared with control subjects and only a minor decrease in bone density of the distal radius. Also, fasting urinary hydroxyproline, a measure of bone resorption, was unchanged with the combination regimen but significantly ($p < 0.05$) increased with nafarelin alone. Further studies involving double-blind, randomized comparison of women treated with a GnRH agonist plus norethindrone and control subjects treated with a GnRH agonist alone are needed.

In conclusion, 5 to 6 years of experience has been gained studying the effects of adding a progesterone or estrogen-progesterone to GnRH agonist therapy for endometriosis or leiomyomata uteri. This experience has provided some important information. The combination of a progestin and a GnRH agonist has a positive impact on the incidence of postmenopausal symptoms and loss of bone density experienced by patients treated with a GnRH agonist alone. The improvements occur without sacrificing the efficacy of the GnRH agonist. However, these benefits may occur at the expense

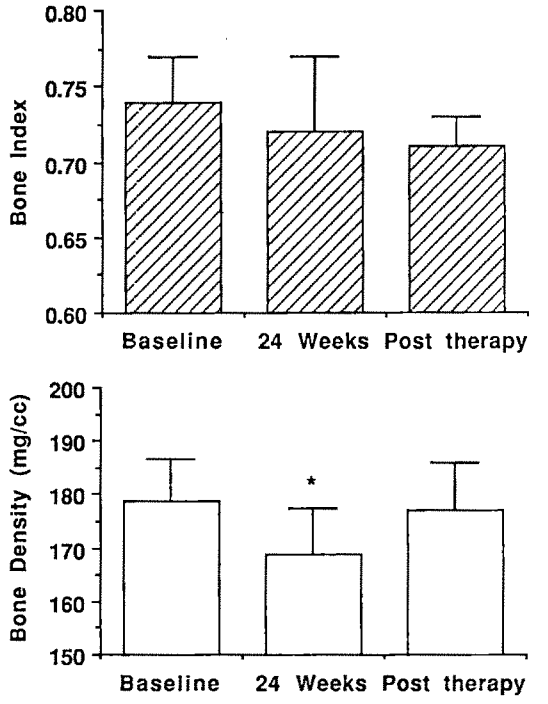


Fig. 3. Bone density measurements of the distal radius *(top)* and lumbar spine *(bottom)* at baseline, after 24 weeks of therapy with a GnRH agonist plus norethindrone, and 24 weeks after therapy. Values are mean ± SE. *$p < 0.05$ vs baseline. (From Surrey ES, Gambone JC, Lu JKH, Judd HL. The effects of combining norethindrone with a gonadotropin agonist in the treatment of symptomatic endometriosis. Fertil Steril 1990;53:620-6. Reproduced with permission of the publisher, The American Fertility Society.)

of an adverse change in the lipid profile; the clinical importance of this change is not known.

Based on these results, the following recommendations can be proposed. In patients with endometriosis in whom a GnRH agonist may be considered, the diagnosis first must be established with laparoscopy. For most patients, initial treatment will be with laser, cautery, or excision. In patients who experience a recurrence of symptoms, consideration should be given to using a GnRH agonist. These agents relieve pain, prevent progression, and delay recurrences of endometriosis.

My recommendation is to add norethindrone, 2.5 mg daily, to the GnRH agonist regimen. This dose of norethindrone will not prevent bone loss but will eliminate vasomotor symptoms that are inherent with GnRH agonist use. Patients who require treatment for longer than 6 months or require a repeat course of therapy with the GnRH agonist should have bone density measurements of the spine or hip obtained before the second course of therapy. The bone density status must be balanced with the patient's severity of symptoms to determine whether to proceed with therapy. If further

therapy is indicated, a higher dose of norethindrone, such as 10 mg daily, could be combined with the GnRH agonist. This higher dose of norethindrone has been found to decrease bone loss in postmenopausal women.[12]

In summary, GnRH agonists are important additions to the management of endometriosis and other reproductive endocrinopathies. Short-term therapy is accompanied by benign but annoying vasomotor symptoms. Long-term therapy is limited by a loss of bone density. Research during the past 5 years has identified alternative treatment regimens that potentially overcome these limitations. Ongoing research will help clarify the role of progestins or estrogen-progestin combinations added to GnRH agonist therapy for the treatment of these conditions.

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Gonadotropin-releasing hormone analogs: Update on new findings

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Clinical trials are under way to investigate the optimal use of nafarelin. In one series of studies, the effect of nasal mucosal inflammation and the concomitant use of nasal decongestants on the nasal absorption of nafarelin was evaluated in women with perennial rhinitis. Neither rhinitis nor concomitant therapy with a long-acting nasal decongestant substantially affected the nasal absorption of nafarelin. Other clinical studies are investigating strategies for second treatment of endometriosis or to improve the safety profile of nafarelin. In one trial, a second 3-month treatment course with nafarelin in patients with recurrent symptoms of endometriosis provided substantial pain relief. In another trial, preliminary information indicates that the addition of norethindrone to nafarelin in patients with endometriosis attenuates bone density changes and menopausal symptoms associated with nafarelin treatment. (Am J Obstet Gynecol 1992;166:757-61.)

Key words: Endometriosis, nafarelin, norethindrone, absorption

The intranasal administration of systemically active therapeutic agents is a novel approach to drug delivery, especially for certain chemical classes of drugs such as peptides. The most important conditions for the successful administration of synthetic peptides by the nasal route are their high intrinsic biologic potency and their ability to enter solutions that are easily absorbed by the nasal mucosa.[1] Nafarelin acetate (further referred to as nafarelin), a decapeptide of the class of gonadotropin-releasing hormone (GnRH) agonistic analogs, is one of the compounds that fulfills these conditions.

Nafarelin is now available for general clinical use for the management of endometriosis. It is one of the most potent, if not the most potent, GnRH analog available, exhibiting 200- to 300-fold greater activity than native GnRH. Therefore it is uniquely suitable for intranasal administration. Previous studies have shown that after intranasal administration with 400 μg/day, nafarelin achieves adequate plasma concentrations to produce the therapeutically desired down-regulation of the pituitary-gonadal system.[1-3]

One of the concerns frequently expressed by practicing gynecologists and infertility specialists is about the adequacy of absorption of nafarelin in patients suffering from rhinitis. Therefore it became important to address this question in a series of clinical trials.

Another question relates to the safety and efficacy of repeated treatment with nafarelin in patients with recurrent symptoms of endometriosis. Although nafarelin has been extensively studied for the management of endometriosis, its current recommended use is limited to 6 months. Therefore we have designed studies in groups of patients who had completed one 6-month treatment course and after at least a 6-month post-treatment pause were started on a second course of nafarelin therapy. In addition, we were interested in identifying alternative treatment strategies that would prevent or reduce the incidence of menopausal symptoms and ameliorate bone density changes associated with the therapeutically induced hypoestrogenism.

This preliminary communication reviews results from current studies addressing these questions, namely, (1) nasal absorption of nafarelin in women with rhinitis, (2) efficacy and safety of retreatment with nafarelin, and (3) therapeutic strategies for prevention of menopausal symptoms and bone metabolism changes that accompany GnRH agonist therapy.

Nasal absorption of nafarelin in women with rhinitis

Results from pharmacokinetic studies in normal volunteers have demonstrated that nafarelin is rapidly absorbed into the systemic circulation after intranasal administration and that the absorption rate is constant over time.[5] To examine the effect of inflammation of the nasal mucosa, such as caused by rhinitis, on the absorption of nafarelin, a clinical trial was undertaken in a group of women with perennial rhinitis. The absorption of nafarelin by nasal mucosa in this group of patients was compared with a group of healthy woman volunteers. Nafarelin, 400 μg, was administered intranasally, and plasma levels of nafarelin were measured over an 8-hour interval.

In women with rhinitis, peak nafarelin plasma levels of 1 ng/ml were achieved within 30 minutes and grad-

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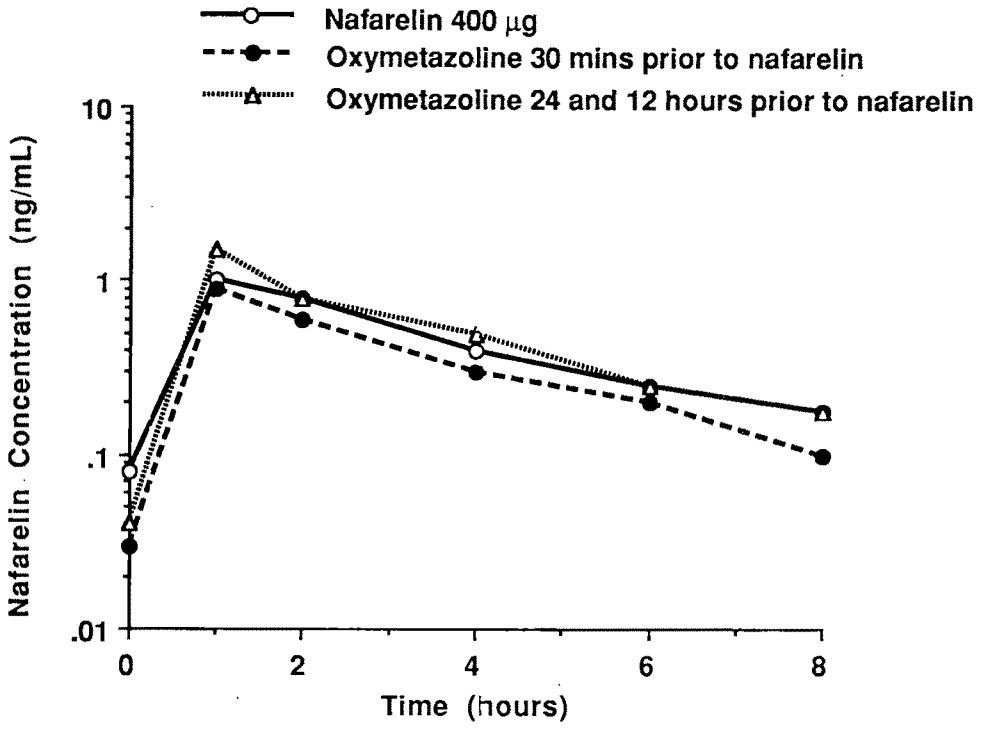


Fig. 1. Plasma nafarelin concentrations after administration of nafarelin, 400 μg, to 12 women. Nafarelin was administered alone, 30 minutes after oxymetazoline, and 12 and 24 hours after oxymetazoline.

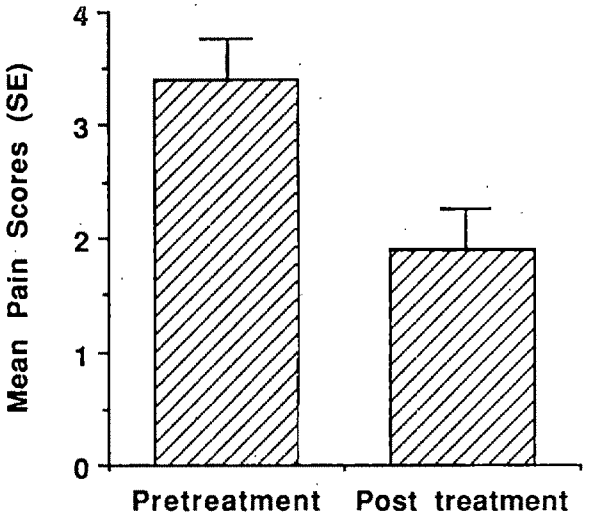


Fig. 2. Mean (±SE) pain scores after 3 months of treatment with nafarelin for recurrent endometriosis.

ually declined with a half-life of about 3 to 4 hours (Fig. 1). This was consistent with the results in normal women; however, nafarelin absorption was slightly enhanced in women with perennial rhinitis compared with the group of normal control subjects. It should be noted that plasma nafarelin levels of 0.5 ng/ml are associated with profound suppression of the pituitary-ovarian system.

In a second trial, the effects of oxymetazoline HCl (Afrin), a commercially available, long-acting decongestant that constricts the small arterioles of the nasal passages, on absorption of nafarelin were evaluated. Oxymetazoline was administered 30 minutes before the administration of 200 μg of nafarelin, and absorption was assessed by repeated measurements of nafarelin plasma levels. In addition, because oxymetazoline is a long-acting product with a duration of action of 12 hours, the absorption characteristics of nafarelin were also studied after the administration of oxymetazoline 12 and 24 hours before the administration of nafarelin.

When oxymetazoline was given 30 minutes before nafarelin, a slight, statistically nonsignificant decrease in nafarelin absorption was noted compared with the nafarelin control group. When oxymetazoline was administered 12 and 24 hours before nafarelin, the absorption of nafarelin was slightly enhanced (Fig. 1).

Thus these results demonstrate that perennial rhinitis and pretreatment with a long-acting nasal decongestant do not appear to affect substantially the nasal absorption of nafarelin. The current recommendations are that nasal decongestants be administered at least 30 minutes after the dose of nafarelin is given.

Efficacy and safety of retreatment with nafarelin

Nafarelin, is currently approved for only one 6-month course for the management of endometriosis.

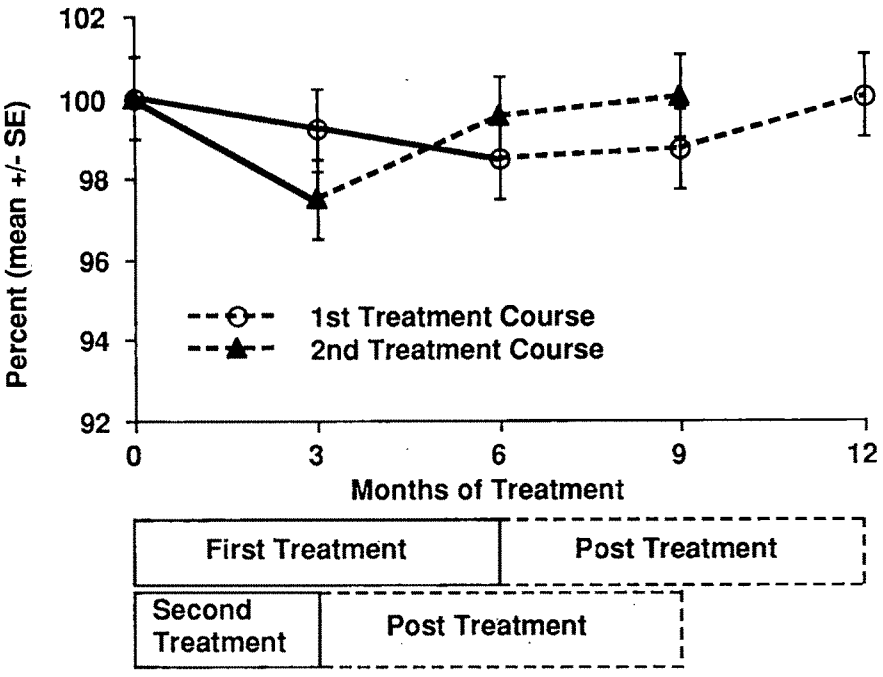


Fig. 3. Changes in lumbar spine bone density assessed by dual-photon absorptiometry during initial 6-month treatment and 6-month follow-up and during 3-month retreatment and 6-month follow-up with nafarelin for recurrent endometriosis.

This is partly because the hypoestrogenic state induced by GnRH agonists is associated with accelerated calcium metabolism in bone tissue and a slight decrease in bone mineral content.[3] The nafarelin-induced hypoestrogenism has little or no effect on bone density changes in compact bone[1,3,4]; rather, it manifests in the trabecular bone of the vertebrae.[3] However, some patients with endometriosis experience a recurrence of symptoms and require treatment with a second course of nafarelin.

In a current clinical trial, patients with recurrent symptoms of endometriosis were treated with a second 3-month course of nafarelin after an initial 6-month course of treatment. The treatment-free period between the first and the second course of therapy was generally 6 months or more. Pain symptoms of endometriosis were evaluated in the same way as in prior studies,[1,3] before the start of nafarelin administration, and at scheduled intervals throughout the study. Changes in bone density of the lumbar spine were measured by dual-photon absorptiometry before the initiation of nafarelin treatment, after completion of the 3-month treatment course, and at the end of the third and sixth months of the posttreatment period. Density of compact bone of the forearm was measured by single-photon absorptiometry. The 3-month regimen was chosen based on results from a previous study that showed that maximal pain relief had already been present at the third month of therapy.[3]

Most patients with endometriosis who received a second course of nafarelin experienced pain relief at the end of the 3-month treatment period. Mean pain scores decreased from about 3.5 before treatment to about 2 after completion of treatment (Fig. 2). As expected, the 3-month retreatment course produced essentially no change in compact bone density as assessed by distal forearm single-photon absorptiometry. Bone density determinations of the lumbar spine showed about a 2.5% to 3% mean decrease during nafarelin therapy; rapid restoration of bone density occurred after withdrawal of therapy (Fig. 3).

Thus it appears that treatment with a second 3-month course of nafarelin for recurrent symptoms of endometriosis affords substantial relief of pain symptoms. In addition, no effect on compact bone was recorded, and the slight loss of lumbar spine density seen during the second 3-month treatment period was rapidly restored after therapy was stopped. These data suggest that a second 3-month treatment course may be effective in patients with recurrent symptoms of endometriosis. Additional trials in a larger patient population will be necessary to confirm these findings.

Evaluation of norethindrone and nafarelin combination therapy on symptoms of hypoestrogenism

We will now discuss the effects of norethindrone on bone density changes and on the incidence of hot

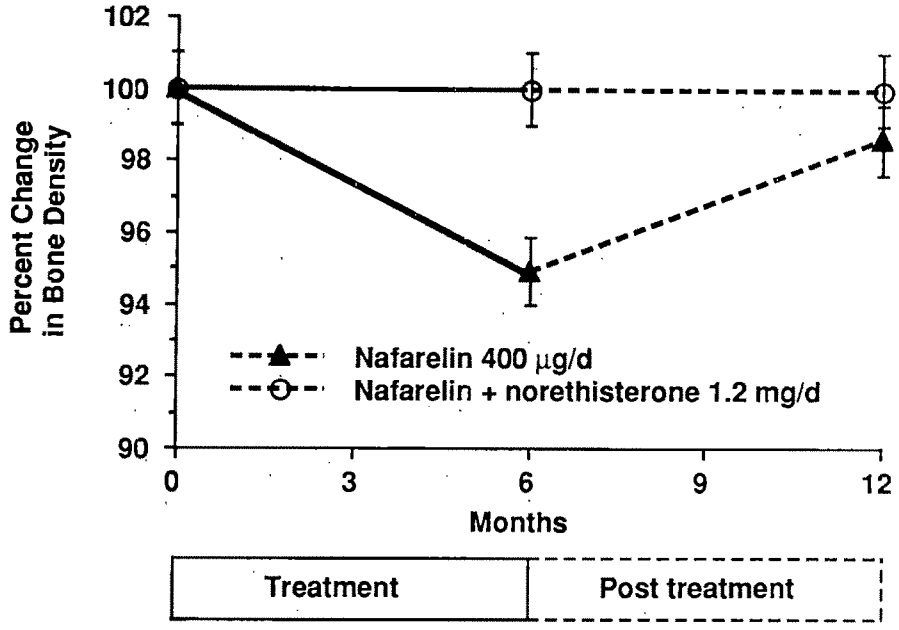


Fig. 4. Changes in lumbar spine bone density assessed by dual-photon absorptiometry with nafarelin, 400 μg/day alone, and combined with norethisterone, 1.2 mg/day. (Modified from Riis et al. Is it possible to prevent bone loss in young women treated with luteinizing hormone–releasing agonists? J Clin Endocrinol Metab 1990;70:920-4. Copyright 1990, Journal of Endocrinology and Metabolism.)

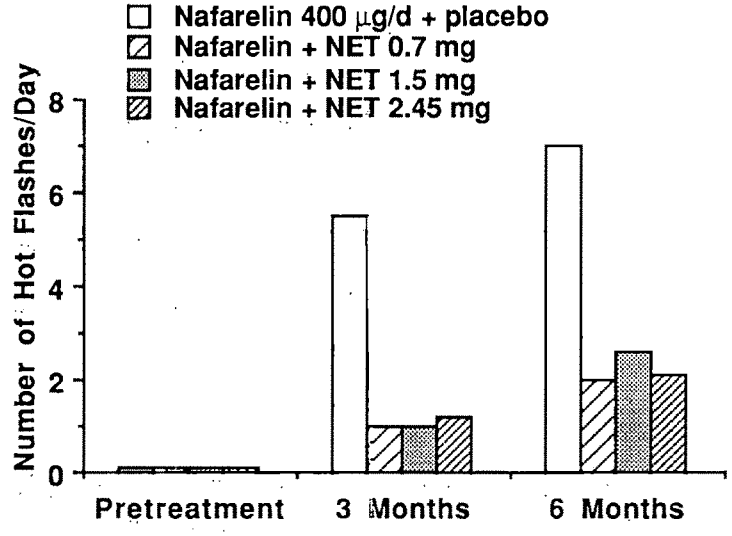


Fig. 5. Average frequency of hot flashes per day in women treated with nafarelin 400 μg/day alone or with combined norethindrone. **NET*, Norethindrone. (Modified from Eldred JM, Thomas EJ. Gynecol Endocrinol 1990;4[suppl 2]:111.)

flashes that are associated with long-term nafarelin treatment.

In the early 1980s, two independent research groups, one led by Mandel et al.[5] and the other by Hart et al.,[6] demonstrated that certain progestogens can counteract the effects of gonadal hormonal deprivation on calcium metabolism in menopausal women. Recently, Riis et al.[4] examined the specific question, does the simultaneous administration of norethindrone and nafarelin exert a bone-sparing effect in patients receiving this therapy for endometriosis. Groups of patients with laparoscopically confirmed endometriosis received either nafarelin alone, 400 μg/day intranasally for 6 months, or norethindrone, 1.2 mg/day orally, in addition to intranasal nafarelin, also for 6 months. Bone density in the lumbar spine was assessed by dual-

photon absorptiometry before treatment, after 6 months of treatment, and after completion of a 6-month posttreatment period. In patients receiving nafarelin alone, bone density in the lumbar spine decreased by an average of 5% at the end of the 6-month treatment period. However, bone density was restored to close to pretreatment levels during the 6-month period after discontinuation of nafarelin therapy. In the group of patients taking 1.2 mg/day of norethindrone in addition to nafarelin, changes in lumbar bone density were attenuated in individual patients, and the average values of trabecular bone density examined at the end of treatment, as well as at the end of the posttreatment observation period, remained unchanged when compared with baseline values (Fig. 4).

The effects of norethindrone on the incidence of hot flashes during nafarelin therapy were studied by Eldred and Thomas.[7] Patients with endometriosis were randomly assigned to treatment with nafarelin, 400 μg/day, and either placebo or norethindrone, 0.7, 1.4, or 2.45 mg daily, and treatment was continued for 6 months. This trial differs from a previously reported trial by Judd et al.[8] in that norethindrone was begun simultaneously with nafarelin.

Patients receiving nafarelin alone averaged about five hot flashes per day after 3 months of treatment, and this increased to about seven episodes daily at the end of the 6-month treatment period (Fig. 5). The addition of norethindrone in the three indicated daily doses substantially decreased the frequency of hot flashes to about two to three per day at the 3- and 6-month evaluations. No dose-response relationship was observed with norethindrone for the doses evaluated in this study.

Thus preliminary data suggest that the combination of nafarelin and norethindrone is associated with beneficial effects on bone density and reduced hypoestrogenic symptoms, such as hot flashes, which are associated with nafarelin treatment. These data are encouraging. Double-blind comparative studies with various doses of progestins administered for 6 months simultaneously with nafarelin are in progress to confirm the results from the described clinical trials.

In conclusion, results from the studies presented here show that nasal absorption of nafarelin is not substantially affected by either perennial rhinitis or concomitant use of long-acting nasal decongestants. Preliminary information from investigations on second treatment with nafarelin for recurrent endometriosis suggests that substantial relief of pain symptoms may be obtained without permanent loss of bone density. Finally, the addition of norethindrone to ameliorate some of the hypoestrogenic effects associated with nafarelin treatment appears to be promising. This research should aid the clinician in selecting optimal therapeutic strategies for the use of nafarelin in the patient with endometriosis.

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Bioavailability of nafarelin in healthy volunteers

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The bioavailability of a single dose of intranasal nafarelin was evaluated in 15 healthy female volunteers. Each subject received a 400 μg intranasal and a 25 μg intravenous dose of nafarelin separated by at least 7 days. Blood samples were obtained frequently after each dose for determination of nafarelin plasma levels. After intravenous administration, time to maximum concentration (T_{max}) was 2 minutes, and maximum serum concentration (C_{max}) was 8.2 ± 2.09 (SD) ng/ml. For intranasal nafarelin, mean T_{max} was 18.4 ± 7.9 minutes (range, 5 to 40 minutes), and C_{max} was 2.04 ± 1.29 ng/ml (range, 0.49 to 5.7 ng/ml). Systemic bioavailability of nafarelin ranged from 1.15% to 5.62% and averaged 2.82% ± 1.23%. Nafarelin's bioavailability is adequate to achieve the desired therapeutic effect because of its inherent high biologic potency and its pharmacokinetic properties. Nafarelin is readily absorbed by the nasal mucosa, and therapeutic blood levels are rapidly achieved and maintained for a prolonged period of time. (AM J OBSTET GYNECOL 1992;166:762-5.)

Key words: Bioavailability, intranasal, nafarelin, volunteers

Nafarelin acetate (nafarelin) is a gonadotropin-releasing hormone (GnRH) agonist recently marketed for the treatment of endometriosis. GnRH agonists are rapidly inactivated by gastrointestinal enzymes after oral administration; therefore alternative routes of administration, including intravenous, intramuscular, and subcutaneous injection, are required to achieve the desired therapeutic effect. Because of the inconvenience and poor patient acceptance of multiple daily injections during long-term therapy, nafarelin is formulated for delivery by the intranasal route.

The purpose of this study was to determine the bioavailability of the commercially available intranasal formulation of nafarelin versus an intravenous formulation of nafarelin.

Material and methods

Subjects. This was an open label, randomized, crossover study in 16 healthy female volunteers. Subjects were between ages 21 and 55 years of age and must have been adequately protected from pregnancy with a method other than hormonal contraception. Subjects were excluded if they had a history of allergies or substance abuse, or if any hormones were taken in the 2 months preceding the study. Subjects were asked to avoid drugs that might interfere with the study for 2 weeks before the study. In addition, they were asked to refrain from any medication for 3 days and from alcohol consumption for 2 days before the study and through its completion. The study protocol was reviewed and approved by an institutional review board. Written informed consent was obtained from each subject.

Study design. Before the study, all subjects underwent a complete physical examination and medical history, and each submitted specimens for hematologic study, clinical chemistry, and urinalysis.

The study was conducted on 2 separate days separated by at least 7 days. Subjects were randomly assigned to receive a single dose of nafarelin, 25 μg intravenously, or 400 μg intranasally. Blood (7.5 ml) was obtained at 0, 2, 5, 10, 20, and 40 minutes and at 1, 2, 4, 6, 8, 12, and 24 hours after the administration of each dose of nafarelin. Serum was separated and frozen for analysis of nafarelin concentrations by radioimmunoassay.[1] The lower quantification limit was 0.04 ng/ml; levels below this limit at 0 and 2 hours were set to zero.

Subjects who received intravenous nafarelin were observed for adverse effects for the first 8 hours after administration of nafarelin. Blood pressure and pulse were measured every 15 minutes for 1 hour. Seven days after the final dose of nafarelin, subjects returned for a repeat physical examination and provided specimens for hematology, clinical chemistry, and urinalysis. Information was solicited about any adverse events or intercurrent complaints.

Statistical and pharmacokinetic analysis. Differences between body weight and vital signs at admission and the final examination were analyzed with the two-sided signed rank test. Changes in vital signs from admission to the start of the intravenous dose and from the intravenous dose to time points beyond were also analyzed with the signed rank test. The signed rank test was used to avoid the assumption of normality required for the repeated measures of analysis of variance. Hematologic and clinical chemistry test results

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were summarized by the change between normal, low, or high as established from the normal limits of the clinical laboratory.

The following pharmacokinetic parameters were determined by noncompartmental methods: time to maximum serum concentration (T_{max}); maximum serum concentration (C_{max}); and serum half-life ($T_{1/2} = Ln2/\beta$). The elimination rate constant (β) was the magnitude of the slope obtained from linear regression of the natural logarithm (Ln) of serum nafarelin concentrations versus time. Total body clearance (CL) was calculated from the equation: CL = dose/(AUC) 0-inf)* × body weight. Volume of distribution (Vd) was calculated from the equation: $Vd = CL/\beta$.

Area under the curve (AUC) was determined from 0 to 8 hours (AUC 0 to 8) and from 0 to infinity. AUC values were obtained by summing the area segments under the serum concentration/time curves. AUC segments between time points with measurable nafarelin levels were calculated by the trapezoidal rule. AUC segments beyond the last measurable level were obtained by extrapolating along an exponential decay curve with the elimination rate constant β.

Systemic bioavailability (F) of intranasal nafarelin was calculated from the percent ratio of AUC 0-inf/dose for the nasal route versus the intravenous route:

$$F(\%) = \frac{AUC_{nasal}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{nasal}} \times 100$$

Dose-normalized serum levels for intranasal nafarelin were calculated by the equation: Normalized serum level = measured value (ng/ml) × 400 μg/estimated dose (μg), where the estimated dose was calculated from the change in the weight of the spray apparatus before and after administration.

Summary statistics and 95% confidence intervals were obtained for all pharmacokinetic parameters. Statistical analysis of these parameters was based on a two-period crossover design.[2] The significance level was 0.05.

Results

Sixteen subjects were randomly assigned to the study. Serum nafarelin data were incomplete in one subject because of interference from an endogenous substance with the radioimmunoassay of samples after both intravenous and intranasal nafarelin. This subject's data were excluded from pharmacokinetic analysis. For the subjects included in the analyses, the average age was 27.7 years (range, 23 to 38 years), the average height was 164.4 cm (range, 151 to 178 cm), and the average weight was 58.8 kg (range, 50 to 70 kg).

Estimated delivery of nafarelin dose. Two sprays from the intranasal apparatus were estimated to deliver 124% ± 51% of the target dose of nafarelin, 400 μg (i.e., 497 ± 203 μg). Serum level data were normalized to the target dose using the estimated dose; however, this did not decrease the variance of calculated pharmacokinetic parameters. Pharmacokinetic parameters calculated from the measured values are presented.

*AUC 0-inf = Area under the curve from 0 to infinity.

Table I. Pharmacokinetic values obtained after administration of single doses of intranasal and intravenous nafarelin to 15 healthy female volunteers*

Parameter	*Intranasal (400 μg)†*	*Intravenous (25 μg)*
T_{max} (min)	18.4 ± 7.9	2.0 ± 0
C_{max} (ng/ml)	2.04 ± 1.29	8.20 ± 2.09
Half-life (hr)	4.4 ± 1.8	3.9 ± 1.4
Clearance (ml/min × kg)	ND‡	0.93 ± 0.17
Volume of distribution (L/kg)	ND	0.31 ± 0.11
AUC 0 to 8 hr (ng × hr/ml)	3.35 ± 1.55	6.74 ± 1.28
AUC 0 to infinity (ng/ml × hr)	4.05 ± 1.82	7.90 ± 1.51
Bioavailability (%)	2.82 ± 1.23	100

*All values are mean ± standard deviation.
†Estimated dose was 497 μg.
‡Not determined.

Pharmacokinetic results. Pharmacokinetic values obtained from intranasal and intravenous administration of nafarelin are shown in Table I. After intravenous administration, T_{max} was 2 minutes, and C_{max} was 8.2 ± 2.09 ng/ml. For intranasal nafarelin, mean T_{max} was 18.4 ± 7.9 minutes (range, 5 to 40 minutes), and C_{max} was 2.04 ± 1.29 ng/ml (range, 0.49 to 5.7 ng/ml). Normalization of the C_{max} values to the 400 μg dose of intranasal nafarelin did not reduce the variability of the data. Mean measured serum levels for intranasal and intravenous nafarelin over the observation period are illustrated in Fig. 1.

The serum nafarelin half-life was similar after intranasal and intravenous administration. For the intranasal dose, half-life ranged from 2.9 to 10 hours (mean, 4.4 ± 1.8 hours). For the intravenous dose, the half-life ranged from 2.2 to 7.8 hours (mean, 3.9 ± 1.4 hours). The total AUC after intravenous administration ranged from 5.04 to 10.56 ng/ml × hours (mean, 7.90 ± 1.51), with a coefficient of variation of 19%. After intranasal administration, the total AUC ranged from 1.37 to 8.15 ng/ml × hours (mean, 4.05 ± 1.82), with a coefficient of variation of 45%. When the total AUC was normalized to the 400 μg dose based on the estimated dose, the total AUC ranged from 1.52 to 7.59 ng/ml × hours; again, normalization did not reduce the variability of data.

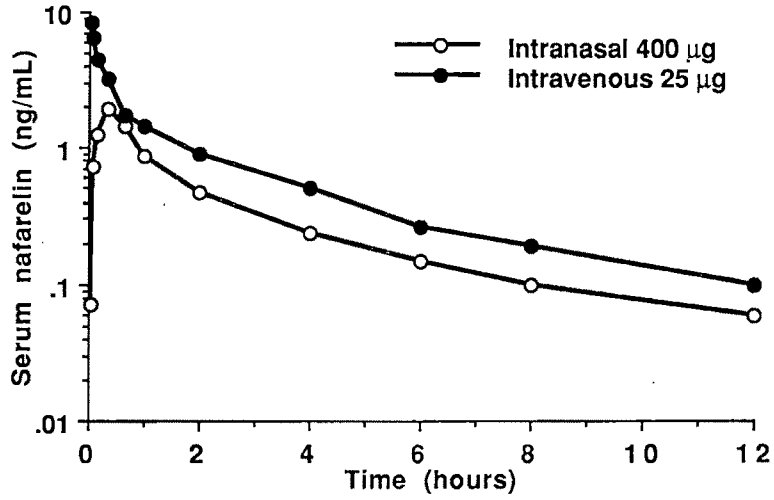


Fig. 1. Mean serum concentrations of nafarelin after a single 400 μg intranasal or 25 μg intravenous dose administered to 15 healthy female volunteers. Estimated delivered intranasal dose was 497 ± 204 μg.

Total body clearance and volume of distribution were determined only for the intravenous nafarelin dose. Clearance ranged from 0.67 to 1.38 ml/min × kg (mean, 0.93 ± 0.17), and volume of distribution ranged from 0.18 to 0.47 L/kg (mean, 0.31 ± 0.11).

The systemic bioavailability of intranasal nafarelin was calculated from measured total AUC values for intravenous and intranasal nafarelin. Systemic bioavailability ranged from 1.15% to 5.62% and averaged 2.82% ± 1.23%.

Comment

The results of this study document the rapid absorption and bioavailability of nafarelin after intranasal administration of a single 400 μg dose. The average T_{max} was 18.4 minutes, and the average C_{max} was 2.04 ng/ml. The average systemic bioavailability was 2.82%.

These results are comparable with those obtained previously in humans and animals.[3, 4] Chan et al.[4] evaluated the bioavailability of intranasal nafarelin relative to subcutaneous nafarelin in six men and observed a mean value of 3.6% ± 1%.[4] A wide interpatient variation in serum levels was noted with intranasal nafarelin in the present study; however, a considerable although lesser variation occurred in serum levels after intravenous administration.

After oral administration, GnRH agonists undergo rapid enzymatic inactivation and are rendered biologically inactive.[5] Thus GnRH agonists must be administered by intravenous, intramuscular, or subcutaneous injection or by nasal, vaginal, or rectal routes.[6] However, intranasal or subcutaneous methods are the only practical and convenient routes of administration for prolonged daily use. Nafarelin is ideal for intranasal administration because of its high biologic potency and long plasma half-life (mean, 4.4 hours). Absorption after intranasal administration is found to be reliable but variable. These properties permit the achievement of desired therapeutic responses by the administration of relatively low doses with a twice-daily regimen.

Intranasal administration of other GnRH agonists is less desirable because of a short half-life or low biologic potency.[7] For instance, tryptorelin, buserelin, and histrelin have biologic potency that is 100-fold greater than native GnRH.[6] However, the plasma half-lives of tryptorelin, buserelin, and histrelin range from 50 to 75 minutes. This necessitates administration of multiple daily doses, when given intranasally to achieve the desired therapeutic response. In contrast, leuprolide has a longer half-life of about 3 hours, but its low biologic potency (15-fold greater than native GnRH) and poor intranasal bioavailability (<5%) require administration of large daily doses to achieve a therapeutic response.[7] One investigator failed to obtain a therapeutic response with 1600 μg/day of intranasal leuprolide in patients with uterine fibroid disease.[8]

The effects of nasal congestion or concurrent use of topical decongestants on the nasal absorption of nafarelin have recently been studied.[9] Perennial rhinitis or use of a nasal decongestant were found to have no significant effect on nasal absorption of nafarelin. These results are consistent with other studies, which showed that nasal absorption of GnRH agonists is unaffected by nasal congestion possibly because of the large surface area of the nasal mucosa.[6] It is recommended that topical decongestants not be used for 30 minutes after intranasal administration of nafarelin.

The results from this study confirm the feasibility of the intranasal route for the administration of nafarelin. Although the systemic bioavailability of intranasal na-

farelin is variable and relatively low, results from large-scale clinical trials have confirmed the clinical efficacy of this formulation for the long-term treatment of endometriosis.[10] Intranasal administration represents a reliable delivery method and convenient dosage route that is well accepted by patients requiring prolonged treatment.

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LETTERS TO THE EDITORS

Intravenous immunoglobulins in postpartum, persistently severe HELLP syndrome: A safe alternative to plasma exchange?

To the Editors: We read with great interest the recent article by Martin et al. (Martin JN Jr, Files JC, Blake PG, et al. Plasma exchange for preeclampsia. I. Postpartum use for persistently severe preeclampsia-eclampsia with HELLP syndrome. AM J OBSTET GYNECOL 1990;162:126-37). The efficiency of plasma exchange in seven well-documented cases actually appears to be convincing because it had already been suggested in some anecdotic cases[1]; however, we would like to draw attention to the risks of plasma-transmitted infections such as non-A, non-B hepatitis; we suggest that intravenous immunoglobulins might be an attractive alternative treatment.

Lian et al.[2] have shown that the immunoglobulin G present in normal adult plasma inhibits the platelet aggregating activity in thrombotic thrombocytopenic purpura plasma and may be partially responsible for the success of plasma infusion therapy in thrombotic thrombocytopenic purpura. The efficiency of immunoglobulins has actually been reported in the treatment of refractory thrombotic thrombocytopenic purpura.[3] Because severe preeclampsia seems to share some common pathophysiologic mechanisms with thrombotic thrombocytopenic purpura[1-4] (as is emphasized by Martin et al.), it appears logical to test the effects of intravenous immunoglobulins in postpartum, persistently severe HELLP (hemolysis, elevated liver enzymes, and low platelet counts) syndrome to avoid the inherent risks of plasma infusion.

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Reply

To the Editors: We appreciate the letter from Pourrat about our article. We, too, have concerns regarding the use of human blood products and the potential for transmission of blood-born infections.

We initially chose plasma as our replacement fluid on the basis of the clinical similarity of the HELLP syndrome and thrombotic thrombocytopenic purpura. Our own studies have demonstrated a marked alteration of the von Willebrand factor multimer composition in this syndrome. Plasma exchange is effective in removing these complexes or adding an antiaggregating component from normal plasma.

Case reports suggesting efficacy of intravenous immunoglobulin preparations are difficult to evaluate in refractory cases of thrombotic thrombocytopenic purpura because of multiple modes of therapy. In occasional patients with chronic relapsing thrombotic thrombocytopenic purpura, intravenous immunoglobulin G has not been effective in reversing the process.[1]

This subgroup of patients (HELLP) with plasma-responsive microangiopathic hemolytic processes may very well be suitable for a study of the potential efficacy of intravenous immunoglobulin G.

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Applicability of the Poiseuille equation to umbilical artery velocimetry

To the Editors: We read with interest the article by Wright and Ridgway (Wright JW, Ridgway RE. Sources of variability in umbilical artery systolic/diastolic ratios: implications of the Poiseuille equation. AM J OBSTET GYNECOL 1990;163:1788-91). They applied the Poiseuille equation to the umbilical artery and found that in their selected study population the systolic/diastolic ratios obtained were normally distributed. Two points should be taken into consideration when analyzing the results:

1. The applicability of the Poiseuille equation to the circulation depends on several conditions[1]: (a) the fluid is homogeneous; (b) the fluid does not slip at the wall; (c) the flow is laminar; (d) the rate of flow is steady and is not subject to acceleration and deceleration; (e) the tube is long compared with the region being studied; and (f) the tube is rigid, and its diameter does not vary with the internal pressure.

In the umbilical artery, because the flow is pulsatile, conditions c, d, and f are not satisfied.

2. With continuous-wave Doppler it is not possi-

ble to locate the sampling site; however, Doppler studies[2-4] have pointed out the effect of sampling site on Doppler ratios and suggested uniform measurement techniques (e.g., choosing the placental end of the cord).

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Reply

To the Editors: We appreciate Degani's interest in our article. We did not intend to imply that the Poiseuille equation is an adequate model for describing the complex flow dynamics of the umbilical circulation. Rather we sought to determine whether the elements in the Poiseuille equation provide clues to factors affecting umbilical artery resistance as measured by the systolic/diastolic ratio.

As noted in our comments, we recognize that continuous-wave Doppler does not permit selection of sampling site along the cord. We initially chose the continuous-wave Doppler system because these devices are commonly available clinically. The studies to which Degani refers had not been published at the time our investigation began. We agree that use of a uniform sampling site should reduce the variability in systolic/diastolic ratios.

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Erythromycin, motilin, and meconium

To the Editors: I read with interest the paper by Eschenbach et al. (Eschenbach DA, Nugent RP, Rao AV, et al. A randomized placebo-controlled trial of erythromycin for the treatment of *Ureaplasma urealyticum* to prevent premature delivery. Am J Obstet Gynecol 1991;164:734-42). Erythromycin is a potent agonist of motilin,[1] which in turn is associated with antenatal passage of meconium by the fetus[2] and fetal distress.[3] Is it therefore possible that long-term therapy with erythromycin for up to 9 weeks of pregnancy could increase the incidence of meconium passage with its attendant sequelae? I note that, although there were no reported differences in the erythromycin-treated group, no mention was made of meconium liquor. It is also of interest that in the same issue of the Journal meconium staining of the amniotic fluid is proposed as a marker for intraamniotic infection (Romero R, Hanaoka S, Mazor M, et al. Meconium-stained amniotic fluid: a risk factor for microbial invasion of the amniotic cavity. Am J Obstet Gynecol 1991;164:859-62). *Ureaplasma urealyticum* is noted to be the most prevalent microorganism found in the patients studied; is it possible that some of these patients had been treated with erythromycin, thereby increasing the incidence of meconium passage via the motilin-like action?

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Reply

To the Editors: We have reviewed the data available on meconium staining of amniotic fluid and aspiration syndrome in the newborn from our erythromycin clinical trial. There is no evidence to support the hypothesis that erythromycin increased either the presence of meconium staining of the amniotic fluid (18.9% in the erythromycin group vs 21.0% in the placebo, $p = 0.37$) or the presence of meconium aspiration syndrome (0.69% in the erythromycin group vs 0.36% in the placebo, $p = 0.68$) in women enrolled in the *Ureaplasma* arm of the clinical trial.

The data from the other arms of the clinical trial (treatment of women with *Chlamydia,* Group B streptococcus, or both with erythromycin) in which an additional 1200 women were enrolled were also reviewed. The results showed a similar lack of evidence (20.1% meconium staining in the erythromycin group vs 18.6% in the placebo, $p = 0.49$; 0.81% meconium aspiration in the erythromycin group vs 1.45% in the placebo, $p = 0.42$) for an erythromycin risk.

The possibility of an association of *Ureaplasma urealyticum* with meconium staining or aspiration syndrome cannot be addressed in the clinical trial reported in our article because the test results of all of the women we studied were positive for the presence of *Ureaplasma.* However, in the same issue of the Journal we also

reported the results of the observational study for women with *Ureaplasma* compared with women without *Ureaplasma* (Carey JC, Blackwelder WC, Nugent RP, et al. Antepartum cultures for ureaplasma urealyticum are not useful in predicting pregnancy outcome. AM J OBSTET GYNECOL 1991;164:728-33). In the observational study no association of *Ureaplasma* with either meconium staining (19.0% in the *Ureaplasma*-positive women vs 19.3% in the *Ureaplasma*-negative women, $p = 0.71$) or aspiration syndrome (1.21% vs 0.89%, $p = 0.12$) was found.

It should be noted that the dose of erythromycin used in our study was lower (333 mg three times each day), but the duration was longer (6 to 10 weeks) than that normally used in pregnancy. The protocol also called for the termination of treatment at the end of 35 weeks' gestation, making it less likely that we would find a lingering effect of erythromycin in term or postterm infants, in whom the risk for meconium-related problems is greatest.

We hope that this additional information will help to allay the concerns raised in Danielian's letter.

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Hypothermia revisited

To the Editors: The "revisit" of Hazan et al. (Hazan J, Maag U, Chessex P. Association between hypothermia and mortality rate of premature infants—revisited. AM J OBSTET GYNECOL 1991;164:111-2) to the association between hypothermia and the mortality rate of premature infants struck us as being flaw-ridden in design and superficial in result. Hypothermia is defined as a core temperature of <35° C. Axillary temperatures approximate core temperature. However, 5 minutes with the mercury bulb deep in the axilla are required to obtain repeatable equilibrium temperatures within 0.1° C.[1] Axillary temperatures should be used only when no obvious thermal problem exists.[2] Thus although axillary temperature monitoring is acceptable for routine clinical use, it is not the optimal method of collecting scientific data about hypothermic infants. Furthermore, the investigators did not indicate whether they used a regular clinical thermometer, which is incapable of measuring temperatures in the hypothermic range, or a special thermometer capable of accurately measuring body temperatures lower than 35° C.

Although Hazan et al. detail the mean admission temperatures of the infants, no information is supplied as to the severity of hypothermia in the cooled infants. Thus included in the group with axillary temperatures <35° C may be severely chilled babies with body temperatures of, say, 30° C, and also minimally cold infants with body temperatures of, say, 34.5° C. The outcome of these two extremes could be very different. No details are supplied regarding other indexes of illness that most certainly must have existed and undoubtedly contributed to the mortality. This is especially true in the >1000 gm birth-weight group.

Controversy exists as to the optimal method of rewarming hypothermic infants: rapid rewarming or the more conventional slow rewarming. We[3] have shown that a combination of neonatal intensive care plus rapid rewarming under a radiant warmer resulted in minimal mortality even in small, severely chilled premature infants. Unfortunately, Hazan et al. give no information regarding their therapeutic approach, which may well have affected the infants' outcome.

In spite of these caveats it should be pointed out that Hazan et al. did demonstrate that hypothermia in infants weighing >1000 gm was associated with increased mortality. In the <1000 gm group the mortality was >50%; the severity of prematurity and its attendant problems outweighed the contribution of hypothermia. Thus to imply that hypothermia is not a contributing factor to mortality in most premature infants is misleading, and the data of Hazan et al. cannot serve as a basis for medical revisionism.

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Reply

To the Editors: Kaplan and Eidelman provide us with the opportunity to expand on our retrospective analysis of body temperature on admission in a neonatal unit. That article was kept succinct to remain within the editorial limits of a brief clinical observation. The working definition of hypothermia ranges widely; we chose a priori a value within that range. In our unit skin temperatures are used in everyday practice to avoid trauma; therefore a skin temperature of <35° C was selected as a cut-off point for hypothermia. Temperatures <35° C were indeed read on an electronic thermometer. Among babies reported as having hypothermia on admission, skin temperatures were in the range 32.0 to 32.9° C in two patients, 33.0 to 33.9° C in six patients, and 34.0 to 34.9° C in the others. Five of eight infants with temperatures <34.0° C died. The association between hypothermia and neonatal mortality was revisited (and not revised) because once again it was verified in premature infants with birth weights ranging from 1000 to 1500 gm. Kaplan and Eidelman call our attention to the possibility that the absence of such an association in the more fragile infants (750 to 999

gm) could be caused by methodologic considerations and the immaturity of the thermogenic capacity. That point is well taken. However, we have to consider that this well-known observation might be merely an association for which it is difficult to prove a cause-and-effect relationship because of numerous confounding factors.

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Preeclampsia, von Willebrand factor antigen, and endothelial cells

To the Editors: I read with interest the article by Roberts et al. (Roberts JM, Taylor RN, Musci TJ, et al. Preeclampsia: an endothelial cell disorder. AM J OBSTET GYNECOL 1989;161:1200-4), the letter by Liston and Kilpatrick (Liston WA, Kilpatrick DC. Preeclampsia—an endothelial cell disorder plus "something else"? AM J OBSTET GYNECOL 1990;163:1365-6), and the reply by Roberts and Taylor (Roberts JM, Taylor RN. Reply. AM J OBSTET GYNECOL 1990;163:1366) regarding preeclampsia as an endothelial cell disorder. As our colleagues in Edinburgh and San Francisco discussed, the cause of preeclampsia may involve immunologic, genetic, or cytotoxic factors. Interest in the endothelial cell and its specific product, von Willebrand factor, are evident. Although I spend little time on obstetrics these days I offer the following observations, which may help us to understand preeclampsia.

The use of von Willebrand factor antigen (vWF:Ag) as a tool in the diagnosis and staging of inflammatory vasculitis is becoming established.[1] Although it may be assumed that very high levels of vWF:Ag are indicative of injury or damage to the endothelium, vWF:Ag levels rise normally in the acute phase response and after exercise.[2, 3] Hypertension in the rat and in the human being can also induce an increase in vWF:Ag, but serum from hypertensive patients was not found to be cytotoxic.[4] The importance of hypertension has recently been underlined[5] with the finding of impaired release of endothelial-derived relaxing factor by endothelial cells from hypertensives.

Immune complexes have long been suspected of some role in vasculitis and, some would say, preeclampsia. With an isotope release assay, serum from patients with rheumatoid vasculitis was cytotoxic to endothelial cells in vitro. Cytotoxicity correlated with immune complexes, vWF:Ag, and inversely with the fourth component of complement. This suggested that high levels of immune complexes and complement activation in these diseases may act to injure endothelial cells, which in turn release vWF:Ag.[6] The cytokine tumor necrosis factor[7] has also been shown to be cytotoxic to endothelial cells in vitro.

I hope some of these issues may be of value in determining the nature of the "something else." It may be that some combination of hypertension, immune complexes, cytokines, and cytotoxic endogenous factors act on endothelial cells to induce the changes that accumulate to cause preeclampsia.

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Reply

To the Editors: We appreciate Blann's interest and comments and are pleased to learn of his studies of endothelial cell injury in hypertension and vasculitis. Certainly this work is very relevant to our hypothesis that maternal vascular endothelial cell injury or activation mediates many of the pathophysiologic features of preeclampsia. Blann's suggestions about von Willebrand factor expression and immune complexes in preeclampsia deserve further comment. Redman et al.[1] were the first to report elevated levels of von Willebrand factor (factor VIII–related antigen) in the plasma of patients with preeclampsia, an observation that has supported the concept of endothelial cell injury or activation in this syndrome. In our initial report,[2] which showed that preeclamptic sera caused increased sodium chromate Cr 51 release from umbilical vein endothelial cells in vitro, we considered the possibility that the observed cytotoxic effect might be mediated by circulating antiendothelial autoantibodies in these patients. Using immunoblotting techniques, we were unable to demonstrate specific antiendothelial autoantibody activities in the sera of women with preeclampsia relative to those of matched controls.

The pitfalls of this approach, however, include the requirements for relatively high-affinity antibodies and their recognition of cognate antigens after denaturation and electrophoretic separation. Indeed, using a more

sensitive whole cell enzyme-linked immunosorbent assay technique, Rappoport et al.[3] did demonstrate the presence of elevated antiendothelial antibody titers in a subpopulation of women with severe preeclampsia. As Blann indicates, similarities between the pathophysiologic features of preeclampsia and rheumatoid vasculitis may provide clues to their causes. The solution of the pathogenesis of preeclampsia and other enigmatic diseases mandates such intellectual contributions from the many disciplines of modern biomedicine.

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Comment on fetal breathing movements after premature rupture of membranes

To the Editors: We read with great interest the article by Roberts et al. (Roberts AB, Goldstein I, Romero R, Hobbins JC. Fetal breathing movements after premature rupture of membranes. AM J OBSTET GYNECOL 1991;164:821-5). Their study used videotapes of real-time ultrasonography of 30 minutes' duration to study fetal breathing and trunk movements in patients of 24 to 34 weeks' gestational age after membranes were ruptured.

In 1988 we published a prospective case-control study on the effect of third-trimester premature rupture of membranes on fetal breathing movements.[1] Our patients' gestational ages were 28 to 41 weeks. During a 30-minute biophysical profile test there was a significant decrease in breathing movements after premature rupture of membranes in otherwise uncomplicated pregnancies (90% vs 65%). Fetal body movements were not changed (97% vs 94%).

Among the patient group in which amnionitis, neonatal infection, or antepartum fetal distress was later detected the incidence of fetal body movement was decreased to 69%. These results agree with the study of Roberts et al.; however, in that study Table I indicated that the time incidence of fetal breathing movements increased with increased fetal age, from 2.6% at 24 to 28 weeks' gestation to 13.3% at 31 to 34 weeks. In our study there was no difference found in the incidence of fetal breathing activity between preterm (28 to 36 weeks) and term pregnancies. This information is especially important because most biophysical profile tests are done at >28 weeks' gestation.

In conclusion, our results complement the study of Roberts et al. in regard to the clinical interpretation of biophysical profile. Fetal breathing movements during 30 minutes of observation decreased or were absent because of premature rupture of the membranes.

It would be interesting to know the incidence of patients with absent breathing movements in the study of Roberts et al.

Asko Kivikoski, MD, and Erol Amon, MD

Departments of Obstetrics and Gynecology, Washington University School of Medicine, and St. Louis Regional Medical Center, 5535 Delmar Blvd., St. Louis, MO 63112

REFERENCE

1. Kivikoski AI, Amon E, Vaalamo PO, Pirhonen J, Kopta MM. Effect of third-trimester premature rupture of membranes on fetal breathing movements: a prospective case-control study. AM J OBSTET GYNECOL 1988;159:1474-7.

Reply

To the Editors: I was interested to read the comments by Kivikoski and Amon. I acknowledge the work they have done in this field and that in 1988 they reported that patients with premature rupture of membranes and no complications had less chance of having 60 seconds of continuous breathing on a biophysical profile than normal controls.

Kivikoski and Amon are concerned about the impact of these findings on interpretation of the biophysical profile. A recent article[1] compared measurement of total fetal activity (fetal breathing plus fetal movement) with biophysical profiles in 64 patients with premature rupture of membranes. All had amniocentesis performed to diagnose infection. Measurement of total fetal activity proved to be better than biophysical profile in detecting intraamniotic infection. The problem seems to be that the sensitivity in the biophysical profile with regard to movements is set at too low a level, and analysis of the amount of movement performed in 30 minutes together with the amount of breathing gives a better measure of fetal well-being.

Kivikoski and Amon asked about the incidence of patients with absent breathing movements in our study. This incidence depends on the gestation, but overall between 24 and 34 weeks, approximately 20% of patients studied with premature rupture of membranes had completely absent fetal breathing movements during a 30-minute recording.

Alistair B. Roberts, MD

Department of Obstetrics and Gynaecology, University of Auckland, National Women's Hospital, Claude Road, Auckland 3, New Zealand

REFERENCE

1. Roberts AB, Goldstein I, Romero R, Hobbins JC. Comparison of total fetal activity measurement with the biophysical profile in predicting intra-amniotic infection in preterm premature rupture of membranes. Ultrasound Obstet Gynaecol 1991;1:36-9.

The natural history of thrombocytopenia associated with preeclampsia

To the Editors: Katz et al. are to be congratulated on their fine article. (Katz VL, Thorp JM Jr, Rozas L,

Bowes WA Jr. The natural history of thrombocytopenia associated with preeclampsia. AM J OBSTET GYNECOL 1990;163:1142-3) Although numerous papers have been written regarding the diagnosis, frequency, treatment, and complications of HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), very few authors have addressed the important issue of the time frame of recovery.

I was surprised by the authors' finding that "bypostpartum day 4, 59 of 61 women had rising counts and all but four women had counts >100,000/mm³." Further, the authors noted that in one of these four patients, sepsis, adult respiratory distress syndrome, and disseminated intravascular coagulation were present and presumably were the cause of the prolonged thrombocytopenia.

In contrast a recent paper by Martin et al.[1] showed that for patients with platelet counts falling below 50,000/mm³, only 80% had recovered to >100,000/mm³ by day 4, and <95% of those with nadirs in platelet count between 50,000 and 100,000 had platelet counts >100,000 by day 4. The experience of Martin et al. is similar to ours; of 41 patients with HELLP syndrome and platelet counts falling below 80,000, eight (19%) had platelet counts below 100,000/mm³ by day 4, and two still had platelet counts below 100,000 by day 6.

Katz et al. do not give the mean platelet count among their 61 patients with HELLP syndrome; among our patients the mean lowest platelet count was 39,000 ± 11,200. The mean peak aspartate aminotransferase level was 521 ± 415 IU/L, and the mean peak lactic dehydrogenase level was 1103 ± 597 IU/L. These figures seem comparable to the values given by Martin et al.[1]

Martin et al.[1] showed a significant difference in recovery time depending on the platelet nadir, with longer recovery times for patients whose platelet counts fell below 50,000. I speculate that the reason for the higher rate of recovery among the patients of Katz et al. as opposed to the series of Martin et al. and our own series may relate to differences in disease severity.

Although it is critically important to search for alternative explanations for thrombocytopenia in all patients with HELLP syndrome, our experience is at odds with the conclusion of Katz et al. that "if a patient with preeclampsia does not have a [platelet] . . . count approaching 100,000/mm³ by postpartum day 4 in the absence of any other pathologic conditions, she is beyond the expected time course of thrombocytopenia associated with preeclampsia."

Val A. Catanzarite, MD, PhD
Sharp Memorial Hospital, San Diego, CA 92123

REFERENCE

1. Martin JN, Blake PG, Lowry S, Perry K, Files JC, Morrison JC. Pregnancy complicated by preeclampsia-eclampsia with the syndrome of hemolysis, elevated liver enzymes, and low platelet count: how rapid is postpartum recovery? Obstet Gynecol 1990;76:737-41.

Response declined

Documentation of high-molecular-weight dextran (Hyskon) solution entering the serum during hysteroscopy

To the Editors: I have read in the surgical literature of cases of noncardiogenic pulmonary edema and coagulopathy[1,2] during hysteroscopy. I provided anesthesia for a patient who had this syndrome,[3] and the cause remained unclear. The recommended dose of high-molecular-weight dextran (Hyskon) solution is <500 ml; however, complications occurred with this volume.

Hyskon hysteroscopy solution continues to be widely used to distend the uterine cavity and visualize its surfaces during diagnostic and therapeutic hysteroscopy. We evaluated the relationship between the amount of Hyskon solution used, the instillation pressures in the uterine cavity, and the serum levels of Hyskon solution. It is my belief that Hyskon solution is absorbed or injected during hysteroscopy, causing pulmonary edema and coagulopathy.

After Institutional Review Board approval and informed consent, 11 class I and II patients scheduled for diagnostic or therapeutic (uterine ablation) hysteroscopy under general anesthesia were studied. Blood samples were obtained to measure Hyskon solution levels. Five patients underwent diagnostic procedures, received a smaller volume of Hyskon solution (<260 ml), and had lower serum Hyskon solution levels of 16.4 ± 22.4 mg/dl. In six ablative procedures, larger volumes of Hyskon solution ranging from 360 to 480 ml, resulted in serum Hyskon solution levels of 1467 ± 913 mg/dl. In all patients mean injection pressures were between 0.5 and 3.9 atm; mean intrauterine pressures were between 10 and 160 mm Hg.

We found much intrauterine Hyskon solution absorption or injection during hysteroscopy. Large intravascular volume expansion occurs with pressurized injection, and medium hypertonicity causes dilutional hyponatremia and anemia. This process may contribute to fluid overload and coagulopathy, especially in hemodynamically compromised patients. During hysteroscopy, only the minimum Hyskon solution volume necessary should be used. Injection pressures should be minimized to reduce intravascular injection. With extensive ablative procedures, a two-stage operation may prevent complications of pulmonary edema and coagulopathy.

Devanand Mangar, MD
Department of Anesthesiology, University of South Florida College of Medicine, Tampa, FL 33612-4799

REFERENCES

1. Jedeikin R, Olsfanger D, Kessler I. Disseminated intravascular coagulation and adult respiratory syndrome: life-threatening complications of hysteroscopy. AM J OBSTET GYNECOL 1990;162:44-5.
2. Leake JF, Murphy AA, Zacur HA. Noncardiogenic pulmonary edema: a complication of operative hysteroscopy. Fertil Steril 1987;48:497-9.
3. Mangar D, Gerson JI, Constantine RM, Lenzi V. Pulmonary edema and coagulopathy due to Hyskon® (32% dextran 70) administration. Anesth Analg 1989;68: 686-7.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

4th Annual Review Course in Obstetrics & Gynaecology, May 6-9, 1992, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA Category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

Office Gynecology, April 20-24, 1992, The Hyatt Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, PA/C, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

June 8, 1992: 2nd International Symposium on Perinatal Asphyxia: Current Concepts and Intrapartum Fetal Surveillance. Presented by The Canadian Medical Protective Association in conjunction with The Society of Obstetricians and Gynaecologists of Canada, at the Westin Bayshore, Vancouver, British Columbia. Contact: Mrs. Joan Beards, C.M.P.A. Education Department, Perinatal Asphyxia Conference, P.O. Box 8225, Ottawa, Ontario, Canada K1G 3H7. Tel.: (613) 236-2100 (ext. 238); fax: (613) 236-5588.

Second Congress of the European Society of Contraception, Astir Palace Complex, "Nafsika" Hotel, Vouliagmemi, Athens, Greece, May 6-9, 1992. Secretariat: c/o G. Creatsas, MD, FACS, 9 Kanari St., Athens 10671, Greece. Tel.: +301-3624367, +301-7770850. Fax: +301-3620484, +301-3229448.

Chicago Area Schools of Medicine Obstetrics and Gynecology Review Course, June 1-6, 1992, Westin Hotel, Chicago, Illinois. 44 hours Category I, 44 Cognates, Formal Learning, ACOG. For information contact: The University of Chicago, Center for Continuing Medical Education, 5841 S. Maryland Ave., Box 139, Chicago, IL 60637. Tel.: (312) 702-1056.

The 3rd Annual Conference on The Prevention of Prematurity and Neonatal Mortality, March 26-28, 1992, at the Westin Peachtree Plaza in Atlanta, Georgia; sponsored by Emory University School of Medicine, with an educational grant from Healthdyne, Inc., Department of Professional Education. Conference Chair: John Huddleston, MD. For further information contact Dr. McEntire at (800) 366-2229 or Emory Office of Continuing Medical Education at (404) 727-5695.

Correction

In the article by Vintzileos et al. entitled "Relationship between biophysical activities and umbilical cord blood gas values" (AM J OBSTET GYNECOL 1991;165:707-13), on page 711, Table V is entitled "Umbilical artery blood gas values associated with compromised biophysical activities"; it should have been "Umbilical vein blood gas values associated with compromised biophysical activities."

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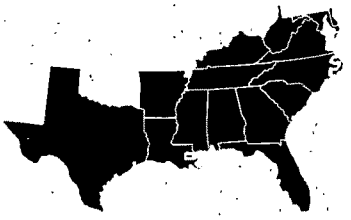
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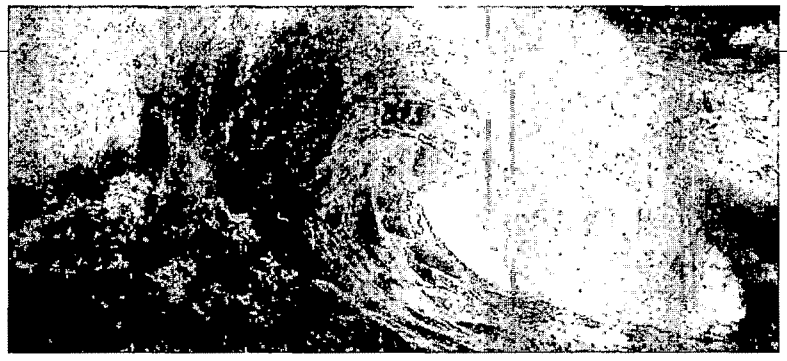
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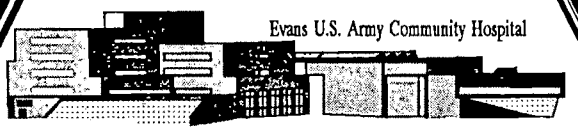


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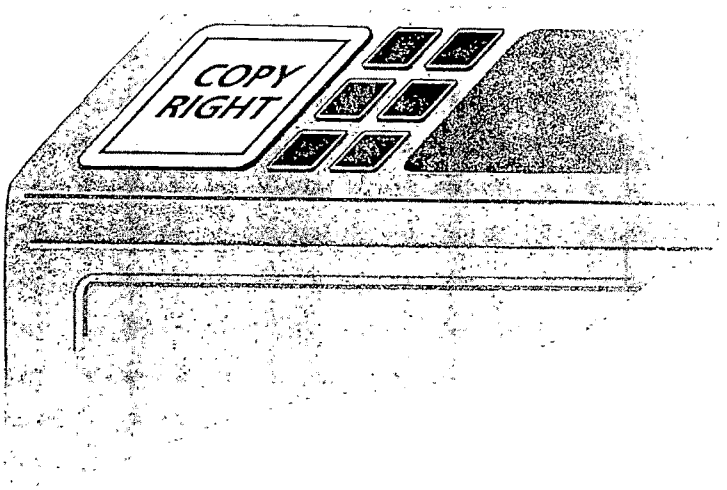
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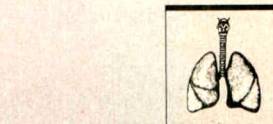
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IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiform, Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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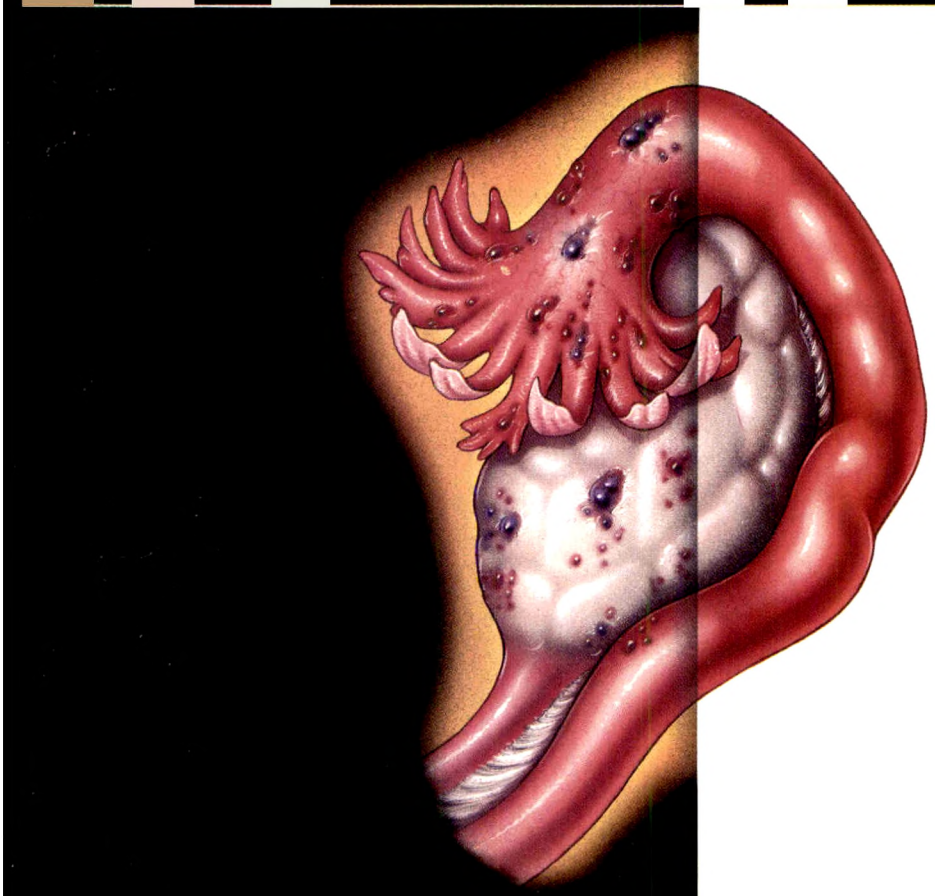
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IN THE MANAGEMENT OF ENDOMETRIOSIS



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Power to suppress estradiol to levels that result in effective relief

- Lowers serum estradiol to near menopausal levels within approximately 1 month, and maintains those levels throughout the 6-month course of therapy.*
- 92% of patients (n = 143) were symptom-free or had only mild symptoms following a 6-month course of therapy.
- Retreatment cannot be recommended since safety data for retreatment are not currently available.

*Pregnancy should be ruled out before therapy with SYNAREL is initiated, and patients should be advised to use a nonhormonal method of contraception when taking SYNAREL.
†There is a small loss in bone density with 6-month treatment, some of which may not be reversible. During one treatment period the bone loss should not be important. ‡Gonadotropin-releasing hormone analog. Please see brief summary of prescribing information on next page.

Control in the management of hypoestrogenic and therapeutic effects

- A small impact on bone,† as well as a predictable and manageable hypoestrogenic profile.
- Lets you terminate treatment immediately should that be required for any reason, unlike the depot form of GnRH‡ therapy.



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NASAL SOLUTION, 2 MG/ML

Setting The Pace In Endometriosis Therapy

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PALO ALTO, CA 94304

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BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve spontaneously or may require drug discontinuation or surgery.

Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the dose. Counsel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients. 3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician. 4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content. 6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption. 7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (± SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

SYNTEX

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April 1991
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†In vitro activity does not necessarily correlate with in vivo effectiveness.

Please see adjacent page for brief summary of prescribing information.

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A BRIEF SUMMARY FOLLOWS.

INDICATIONS AND USAGE
TREATMENT

CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Proteus mirabilis,* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K. pneumoniae*), *E. coli, Proteus mirabilis,* and *Serratia marcescens**.

Skin and Skin Structure Infections due to *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *Escherichia coli, Klebsiella pneumoniae, Peptococcus niger**, *Peptostreptococcus* species.

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *Streptococcus agalactiae, E. coli, Proteus mirabilis, Neisseria gonorrhoeae,* Bacteroides species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus niger* and *Peptostreptococcus* species).

Cefotetan, like other cephalosporins, has no activity against *Chlamydia trachomatis.* Therefore, when cephalosporins are used in the treatment of pelvic inflammatory disease, and *C. trachomatis* is one of the suspected pathogens, appropriate antichlamydial coverage should be added.

Intra-abdominal Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Streptococcus species* (excluding enterococci), *Bacteroides* species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*) and *Clostridium* species*.

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic *in vitro* against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH CEFOTAN IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTETAN DISODIUM, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS-HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO CEFOTAN OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including cefotetan, and may range from mild to life-threatening. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *Clostridium difficile.*

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: The administration of CEFOTAN may result in a false-positive reaction for glucose in the urine using Clinitest®†, Benedict's solution, or Fehling's solution. It is recommended that glucose tests based on enzymatic glucose oxidase be used.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Pregnancy: Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy. Those appearing in italics have been reported during postmarketing experience.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700); *pseudomembranous colitis.* Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. (See WARNINGS.)

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300); *agranulocytosis, hemolytic anemia, leukopenia, thrombocytopenia,* and *prolonged prothrombin time.*

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700); *anaphylactic reactions.*

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

Miscellaneous: *Fever*

In addition to the adverse reactions listed above which have been observed in patients treated with cefotetan, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics: urticaria, pruritis, Stevens-Johnson syndrome, erythema multiforme, toxic epidermal necrolysis, vomiting, abdominal pain, colitis, superinfection, vaginitis including vaginal candidiasis, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, increased BUN, increased creatinine, elevated bilirubin, pancytopenia, and neutropenia.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see DOSAGE AND ADMINISTRATION and OVERDOSAGE). If seizures associated with drug therapy occur, the drug should be discontinued. Anticonvulsant therapy can be given if clinically indicated.

DOSAGE AND ADMINISTRATION
TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Skin & Skin Structure		
Mild-Moderate[a]	2 grams	2 g every 24 hours IV 1 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6 grams[b]	3 g every 12 hours IV

[a] *K. pneumoniae* skin and skin structure infections should be treated with 1 or 2 grams every 12 hours IV or IM.

[b] Maximum daily dosage should not exceed 6 grams.

If *C. trachomatis* is a suspected pathogen in gynecologic infections, appropriate antichlamydial coverage should be added, since cefotetan has no activity against this organism.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. See FULL PRESCRIBING INFORMATION for a complete description.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

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References: 1. Walker CK, Landers DV, Ohm-Smith MJ, et al. Comparison of cefotetan plus doxycycline with cefoxitin plus doxycycline in the inpatient treatment of acute salpingitis. *Sex Trans Dis.* 1991;18:119-123. **2.** McGregor JA, Gordon SF, Krotec J, Poindexter AN. Results of a randomized, multicenter, comparative trial of a single dose of cefotetan versus multiple doses of cefoxitin as prophylaxis in cesarean section. *Am J Obstet Gynecol.* 1988;158:701-706. **3.** Carver P, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986(suppl):11-14. **4.** Ohm-Smith MJ, Sweet RL, Hadley WK. In vitro activity of ceftuperazone and other antimicrobial agents against isolates from the female genital tract. *Antimicrob Agents Chemother.* 1985;27:958-960. **5.** Data on file, Stuart Pharmaceuticals, Wilmington, Delaware. **6.** Souza Dias MB, Jacobus NV, Tally FP, Gorbach SL. Activity of cefotetan against anaerobic bacteria. *Diagn Microbiol Infect Dis.* 1986;4:359-363. **7.** Wexler HM, Finegold SM. In vitro activity of cefotetan compared with that of other antimicrobial agents against anaerobic bacteria. *Antimicrob Agents Chemother.* 1988;32:601-604. **8.** Hennessey TD. Cefotetan: a compilation of susceptibility data of clinical isolates. In: Lode H, Periti P, Strachan CJL, eds. *Cefotetan: A Long-Acting Antibiotic for Polymicrobial Infections.* New York, NY: Churchill Livingstone; 1985:14-22. **9.** Centers for Disease Control. 1989 sexually transmitted diseases treatment guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

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Superficial laser vulvectomy. V. Surgical debulking is enhanced by adjuvant systemic interferon 815
Richard Reid, MD, Mitchell D. Greenberg, MD, David J. Pizzuti, MD, Katherine H. Omoto, MS, Lisa H. Rutledge, BA, and Whaijen Soo, MD, PhD
Detroit and Southfield, Michigan, and Nutley, New Jersey

In conjunction with surgical debulking by extended laser ablation, the use of adjuvant subcutaneous alpha interferon reduced the risk of early postoperative recurrence by about two thirds.

Therapeutic amniocentesis in the twin-twin transfusion syndrome appearing in the second trimester of pregnancy 820
N.J. Saunders, MD, R.J.M. Snijders, MSc, and K.H. Nicolaides, MD
London, England

Survival rate (37%) in 19 twin pregnancies with acute second-trimester polyhydramnios treated with amniocentesis was related to volume of amniotic fluid drained and intertwin disparity in size.

Immunohistochemical detection of the multi-drug-resistance marker P-glycoprotein in uterine cervical carcinomas and normal cervical tissue 825
José Schneider, PhD, Thomas Efferth, PhD, Jürgen Mattern, MD, Francisco-Javier Rodriguez-Escudero, PhD, and Manfred Volm, PhD
Bilbao, Spain, and Heidelberg, Germany

The multi-drug-resistance marker P-glycoprotein was detected by immunohistochemical examination in 10 of 11 uterine cervical carcinomas and all normal controls.

A randomized trial of prostaglandin E_2 in a controlled-release vaginal pessary for cervical ripening at term 830
Frank R. Witter, MD, Laura E. Rocco, MS, and Timothy R.B. Johnson, MD
Baltimore, Maryland

A new, controlled-release vaginal prostaglandin E_2 pessary has been shown to be efficacious for cervical ripening at term.

The resolution of chronic pelvic pain after normal laparoscopy findings 835
Philip N. Baker, DM, and E. Malcolm Symonds, MD
Nottingham, England

The symptoms of a majority of 60 patients with chronic pelvic pain resolved after laparoscopy without treatment.

Prostaglandin E_2 for induction of labor in patients with premature rupture of membranes at term 836
Deborah A. Ray, MD, and Thomas J. Garite, MD
Santa Clara and Orange, California

Prostaglandin E_2 can be used successfully to induce labor after premature rupture of membranes with lower maternal infectious morbidity and greater ease of administration, when compared with oxytocin.

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Inviting all members of ACOG to
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NEW PERSPECTIVES IN THE MANAGEMENT OF THE PREGNANT WOMAN WITH EPILEPSY

A Breakfast Symposium for Members of ACOG

Monday, April 27, and Tuesday, April 28

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Registration and Breakfast beginning at 5:30 AM

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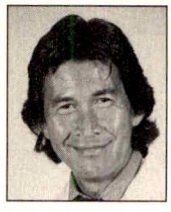
Registration

All members of the American College of Obstetricians and Gynecologists will receive an invitation and registration form by mail. Please complete and return that form as soon as possible. Space is limited and registrations will be accepted on a first-come, first-served basis. If, however, you do not preregister, you are welcome to do so immediately before the meeting on either Monday, April 27, or Tuesday, April 28.

Acknowledgment

This symposium is made possible through an educational grant from Parke-Davis, Division of Warner-Lambert Company.

Faculty and Program



Neurology Consensus Guidelines: Preconception Counseling, Management, and Care

Antonio V. Delgado-Escueta, MD, Co-Chairman
Professor of Neurology
UCLA School of Medicine
Director
California Comprehensive Epilepsy Program, and
VA Southwest Regional Epilepsy Center
West Los Angeles, California



The Challenge to the Obstetrician: Prenatal Diagnosis and Management

Khalil M.A. Tabsh, MD, Co-Chairman
Professor and Chief
Obstetrics and Gynecology
UCLA Center for the Health Sciences
Los Angeles, California



Current Research on Major Anomalies Associated with Epilepsy and Antiepileptic Agents

Dick Lindhout, MD
Professor of Clinical Genetics and Teratology
Klinische Genetica
Erasmus Universiteit
Rotterdam, Netherlands



Recent Findings on Minor Anomalies and Psychomotor Development

Mark S. Yerby, MD, MPH
Clinical Associate Professor of Public Health and Preventative Medicine
Oregon Health Sciences University, and
Director of Research
Oregon Comprehensive Epilepsy Program
Good Samaritan Hospital
Portland, Oregon

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The cavitational ultrasonic surgical aspirator for cytoreduction in advanced ovarian cancer 843

Peter G. Rose, MD

Worcester, Massachusetts

Over a 24-month period the cavitational ultrasonic surgical aspirator was used for cytoreduction in 48% of patients with advanced ovarian cancer.

Does oxytocin augmentation increase perinatal risk in primigravid labor? 847

David J. Cahill, MB, Peter C. Boylan, MAO, and Colm O'Herlihy, MD

Dublin, Ireland

Oxytocin augmentation of spontaneous labor in 14,119 nulliparous women did not increase the incidence of asphyxial perinatal death or neurologic abnormality among their infants.

The frequency of breech presentation by gestational age at birth: A large population-based study 851

Durlin E. Hickok, MD, MPH, Diane C. Gordon, MS, MPH, John A. Milberg, MPH, Michelle A. Williams, ScD, and Janet R. Daling, PhD

Seattle, Washington

Breech presentation was found to be less frequent at each gestational age from a study of birth records, when compared with prior studies that used antenatal ultrasonography.

Ovarian carcinoma of low malignant potential, infertility, and induction of ovulation—Is there a link? 853

Gary L. Goldberg, MD, and Carolyn D. Runowicz, MD

Bronx, New York

We report three cases of ovarian carcinoma of low malignant potential associated with infertility and ovulation induction in patients <35 years old.

Maternal and fetal cardiovascular responses to strenuous bicycle exercise 854

Marieke B. van Doorn, MD, Fred K. Lotgering, MD, PhD, Piet C. Struijk, BSc, Jan Pool, MD, PhD, and Henk C.S. Wallenburg, MD, PhD

Rotterdam, The Netherlands

Pregnancy does not markedly modulate the maternal electrocardiogram or blood pressure response to strenous bicycle exercise; prior exercise does not markedly affect fetal heart rate.

Is trichomoniasis often associated with bacterial vaginosis in pregnant adolescents? 859

Janine A. James, MD, Jessica L. Thomason, MD, Sheldon M. Gelbart, PhD, Peter Osypowski, MT(ASCP), Paul Kaiser, RN, CNM, and Lisa Hanson, RN, CNM

Milwaukee, Wisconsin

Pregnant adolescents with *Trichomonas vaginalis* often have signs and symptoms of bacterial vaginosis, but the two conditions can be differentiated.

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Laparoscopic radical hysterectomy with paraaortic and pelvic node dissection 864

Camran R. Nezhat, MD, Matthew O. Burrell, MD, Farr R. Nezhat, MD, Benedict B. Benigno, MD, and Charles E. Welander, MD

Macon and Atlanta, Georgia

Laparoscopic radical hysterectomy and paraaortic and pelvic lymphadenectomy are reported.

Emergency room use of transvaginal ultrasonography by obstetrics and gynecology residents 866

Ilan Timor-Tritsch, MD, Susan Greenidge, MD, Dahlia Admon, MD, and M. Lynne Reuss, MD

New York, New York

The emergency use of transvaginal ultrasonography by obstetrics and gynecology residents shortened the time to establish a diagnosis in patients seen in the emergency room.

Ultrasonographic dating of pregnancy causes significant errors in Down syndrome risk assessment that may be minimized by use of biparietal diameter–based means 872

Timothy M. Reynolds, MB, ChB, Michael D. Penney, MD, and Howard Hughes, MSc

Cardiff and Newport, Wales

If ultrasonographically derived gestational week is used to define multiples of the median for Down syndrome risk assessment, the calculated risks may be incorrect.

Infarction of the uterus from subacute incomplete inversion 878

Mary S. Romo, MD, David A. Grimes, MD, and Philip O. Strassle, MD

Los Angeles, California

Intravenous administration of sodium fluorescein dye confirmed the intraoperative diagnosis of necrosis of the uterus, which led to the decision for hysterectomy.

The primary application of three-dimensional ultrasonography in obstetrics 880

Hong-Chang Kuo, MD, Fong-Ming Chang, MD, Chun-Hsein Wu, MD, Bor-Lin Yao, MD, and Chi-Hong Liu, MD

Tainan, Taiwan

We suggest that three-dimensional ultrasonography provides more information for clinical diagnosis and understanding of the fetal anatomy than conventional two-dimensional ultrasonography has done.

Cellular immunity of patients with recurrent or refractory vulvovaginal moniliasis 887

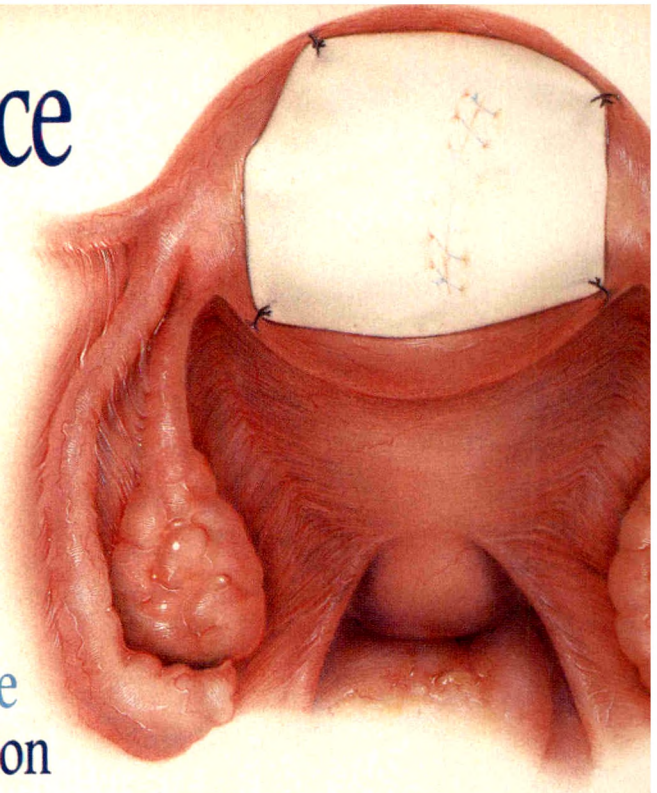
Ignatius W. Fong, MD, Paul McCleary, MD, and Stanley Read, MD

Toronto, Ontario, Canada

Cellular immunity was studied in 73 patients with recurrent vaginal moniliasis and 37 healthy control subjects; most patients were found to have normal cellular immunity.

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Invariant Performance in Peritoneal Reconstruction of Myomectomy Sites

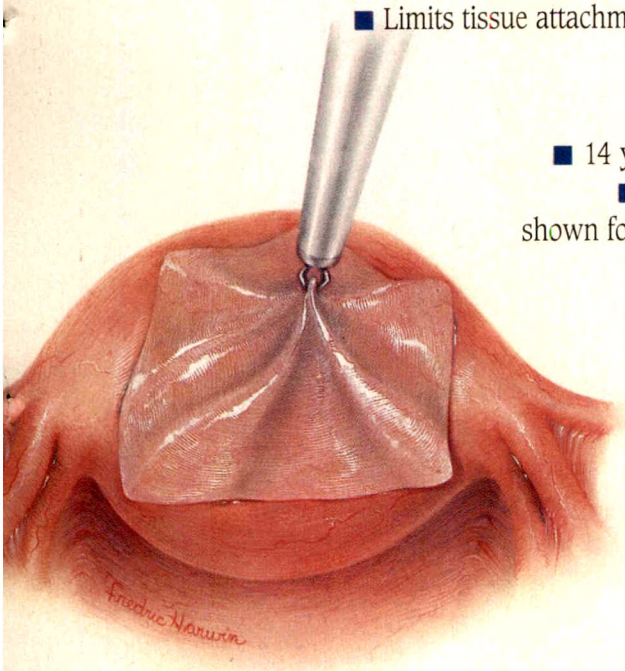


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Validity of postnatal assessments of gestational age: A comparison of the method of Ballard et al. and early ultrasonography 891
Greg R. Alexander, MPH, ScD, Francois de Caunes, MD, MPH, Thomas C. Hulsey, MSPH, ScD, Mark E. Tompkins, PhD, and Marilee Allen, MD
Minneapolis, Minnesota, Guadeloupe, French West Indies, Charleston and Columbia, South Carolina, and Baltimore, Maryland

This study investigates the accuracy of postnatal gestational age assessments to predict preterm, term, postterm, and small-for-gestational-age infants with early ultrasonography used as the gold standard.

Diagnosis of cervical change in pregnancy by means of transvaginal ultrasonography 896
Gary M. Joffe, MD, Gerardo O. Del Valle, MD, Luis A. Izquierdo, MD, George J. Gilson, MD, James F. Smith, MD, Molly S. Chatterjee, MD, and Luis B. Curet, MD
Albuquerque, New Mexico

Endovaginal ultrasonographic assessment of the cervix may provide an objective basis for the diagnosis of cervical incompetence.

Contraceptive compliance with a levonorgestrel triphasic and a norethindrone monophasic oral contraceptive in adolescent patients 901
Elizabeth R. Woods, MD, MPH, Estherann Grace, MD, Kathryn Klein Havens, MD, James L. Merola, PhD, and S. Jean Emans, MD
Boston, Massachusetts

Contraceptive compliance for a levonorgestrel triphasic pill was compared with that for a norethindrone monophasic pill in adolescent patients at a clinic and in a private practice.

Prenatal ultrasonographic diagnosis of fetal scrotal inguinal hernia 907
Israel Meizner, MD, Arie Levy, MD, Miriam Katz, MD, Tzippi Simhon, and Marek Glezerman, MD
Beer-Sheva, Israel

A rare case of in utero ultrasonographic diagnosis of fetal scrotal inguinal hernia is presented.

Uterine and umbilical flow velocity waveforms in normotensive and hypertensive subjects during the angiotensin II sensitivity test 910
Risto U. Erkkola, MD, PhD, and Jouko P. Pirhonen, MD
Turku, Finland

The increase in the systolic/diastolic ratio in the uterine artery as a result of angiotensin II was similar in normotensive and hypertensive subjects, yet the recovery was slower in hypertensive ones.

Patterns of failure of bulky-barrel carcinomas of the cervix 916
Deborah L. Coleman, MD, Donald G. Gallup, MD, Hugh D. Wolcott, MD, Luther B. Otken, MD, and Richard J. Stock, MD
Augusta, Georgia, and Portsmouth, Virginia

An analysis of patients with bulky-barrel cervical lesions indicates recurrences are frequently outside the pelvis, and adjunctive chemotherapy may be beneficial to some patients who have hysterectomy after irradiation.

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Normal fetal Doppler inferior vena cava, transtricuspid, and umbilical artery flow velocity waveforms between 11 and 16 weeks' gestation 921
Juriy W. Wladimiroff, MD, PhD, Tjeerd W.A. Huisman, MD, Patricia A. Stewart, PhD, and Th. Stijnen, PhD
Rotterdam, The Netherlands

At 11 to 16 weeks' gestation there is a reduction in fetal inferior vena cava retrograde flow that may be determined by rising cardiac compliance or ventricular relaxation rate.

Maternal glucose intolerance and the subcutaneous terbutaline pump 925
Connie Lindenbaum, MD, Jack Ludmir, MD, Frann B. Teplick, MSN, RNC, Arnold W. Cohen, MD, and Philip Samuels, MD
Philadelphia, Pennsylvania

Use of the subcutaneous terbutaline pump in the treatment of premature labor does not appear to increase the incidence of maternal glucose intolerance.

Hydrops fetalis associated with congenital myotonic dystrophy 929
Ashraf M. Afifi, MD, Asha Rijhsinghani Bhatia, MD, and Fabian Eyal, MD
Baltimore, Maryland

A case report of hydrops fetalis associated with congenital muscular dystrophy is presented; congenital muscular dystrophy can be associated with fetal hydrops and should be included among the various causes of nonimmune hydrops fetalis.

Vaginal injury resulting from sliding down a water chute 930
Jacob Niv, MD, Joseph B. Lessing, MD, Joseph Hartuv, MD, and M. Reuben Peyser, MD
Tel Aviv, Israel

This article provides a description of a woman who sustained a vaginal laceration after sliding down a water chute while using a vaginal tampon; the potential mechanism is discussed.

Fetal urine biochemistry in the assessment of obstructive uropathy 932
K.H. Nicolaides, MD, H.H. Cheng, MD, R.J.M. Snijders, MSc, and C.F. Moniz, MD
London, England

In 60 fetuses with obstructive uropathy the best predictors of renal function were the urinary concentrations of calcium and/or sodium.

Characteristics of three vaginal flora patterns assessed by Gram stain among pregnant women 938
Sharon L. Hillier, PhD, Marijane A. Krohn, PhD, Robert P. Nugent, PhD, and Ronald S. Gibbs, MD, for the Vaginal Infections and Prematurity Study Group
Seattle, Washington, Bethesda, Maryland, and Denver, Colorado

There are three vaginal flora patterns in pregnant women associated with different clinical and microbiologic characteristics.

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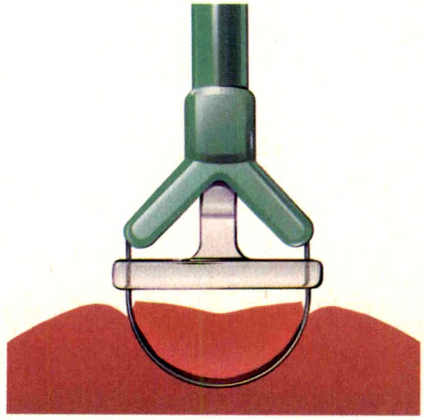


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*PATENTS PENDING

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Preterm premature rupture of membranes: Results of expectant management in patients with cervical cultures positive for group B streptococcus or *Neisseria gonorrhoeae* 945

George Larry Maxwell, MD, and William J. Watson, MD

Chapel Hill, North Carolina

In patients with preterm premature rupture of membranes and a positive cervical culture who are treated with prophylactic antibiotics, there is an increased risk of neonatal pneumonia.

Comparison of transdermal and oral estrogen-progestin replacement therapy: Effects on serum lipids and lipoproteins 950

David Crook, PhD, Michael P. Cust, MB, Kevin F. Gangar, MB, Melek Worthington, MSc, Timothy C. Hillard, BM, John C. Stevenson, MB, Malcolm I. Whitehead, MB, and Victor Wynn, MD

London, England

Transdermal administration of postmenopausal estrogen-progestin alters plasma lipoprotein risk factors for cardiovascular disease.

Oral contraceptive type and functional ovarian cysts 956

Stephan F. Lanes, PhD, Brenda Birmann, BA, Alexander M. Walker, MD, DrPH, and Suanne Singer, BA

Chestnut Hill and Boston, Massachusetts, and Augusta, Maine

The preventive effect of oral contraceptives on symptomatic functional ovarian cysts is greater for monophasic pills—especially high-dose pills—than for multiphasic pills.

Plasma endothelin levels in preeclampsia: Elevation and correlation with uric acid levels and renal impairment 962

Barbara A. Clark, MD, Lisa Halvorson, MD, Benjamin Sachs, MD, and Franklin H. Epstein, MD

Boston, Massachusetts

Plasma endothelin levels were elevated in women with preeclampsia as compared with normal pregnant and nonpregnant women and correlated closely with markers of disease severity.

Latex anaphylaxis in an obstetrics and gynecology physician 968

M. Dwight Chen, MD, Jeffrey S. Greenspoon, MD, and Toni L. Long, MD

Los Angeles, California

Latex anaphylaxis caused by surgical gloves occurred during an obstetric emergency and necessitated treatment of the physician.

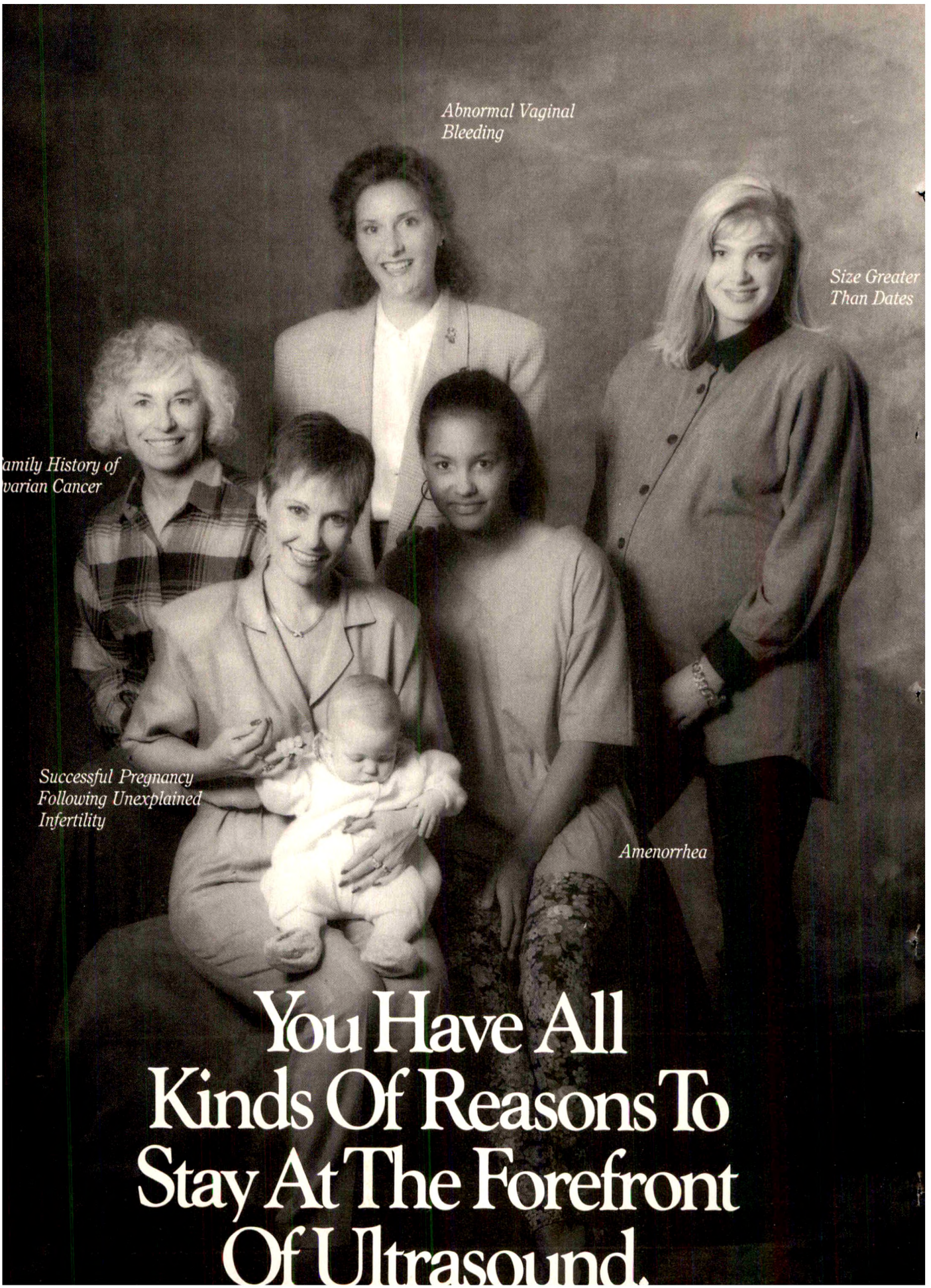
Does amniotic fluid analysis reflect acid-base balance in fetal blood? 970

D.L. Economides, MD, P. Johnson, MA, and I.Z. MacKenzie, MD

Oxford, England

Sampling of human fetal umbilical blood and amniotic fluid in normal and red blood cell isoimmunized pregnancies failed to show significant correlations in acid-base values except between amniotic fluid and umbilical venous P_{CO_2}.

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Abnormal Vaginal Bleeding
Size Greater Than Dates
amily History of varian Cancer
Successful Pregnancy Following Unexplained Infertility
Amenorrhea
You Have All Kinds Of Reasons To Stay At The Forefront Of Ultrasound.

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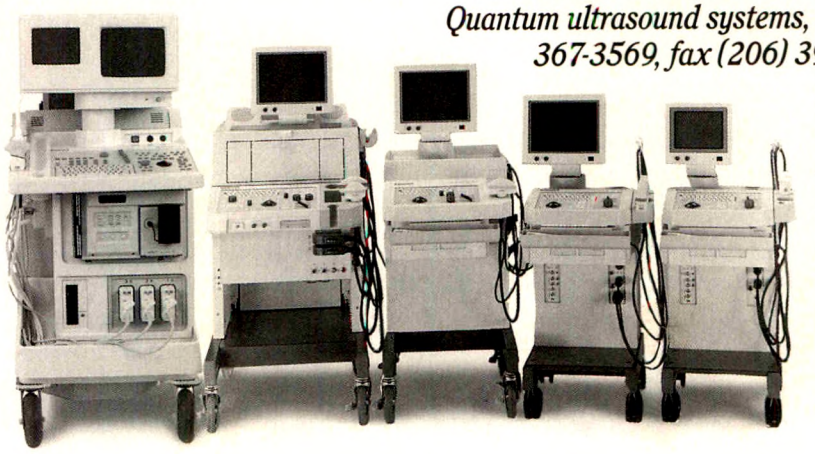
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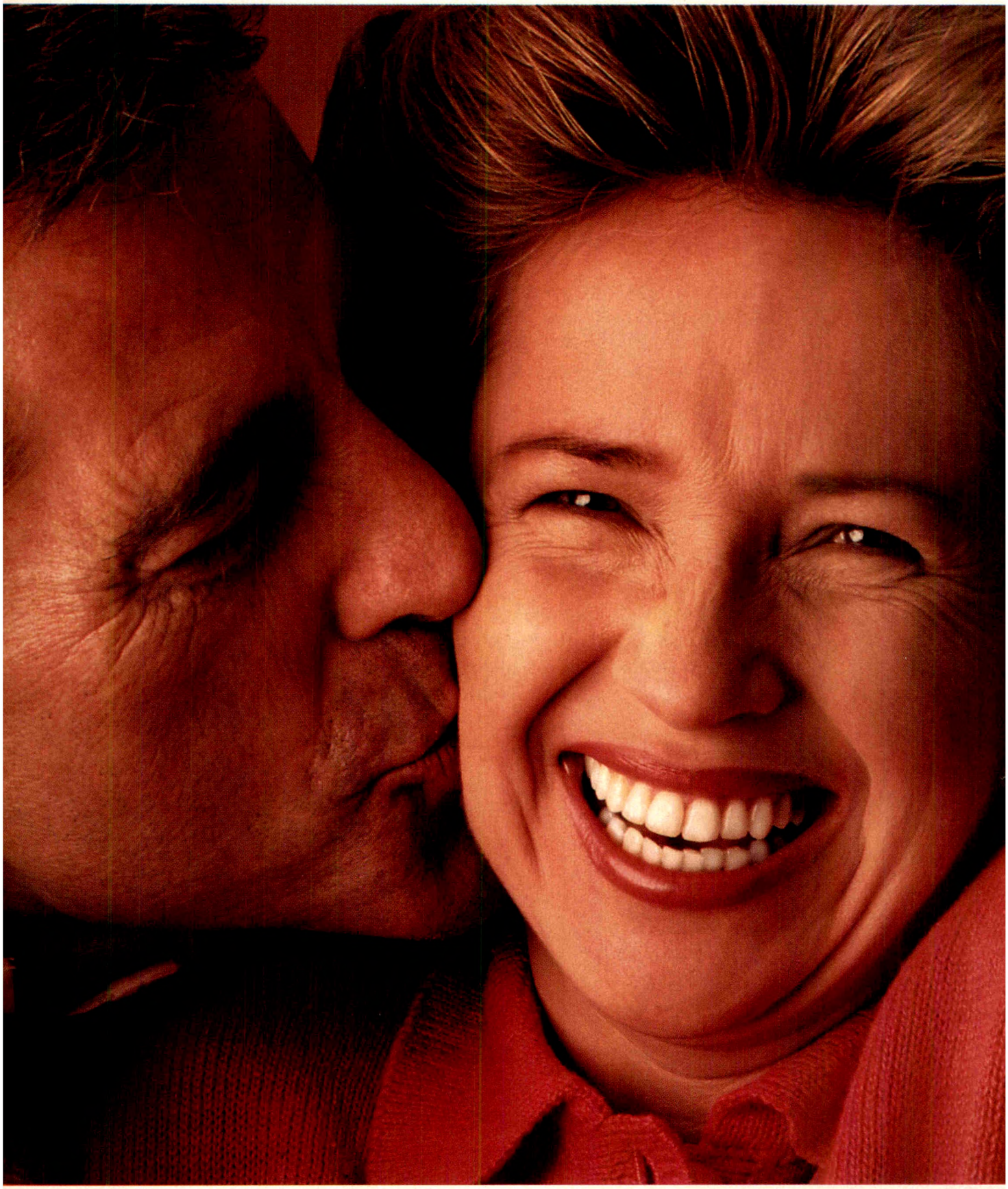
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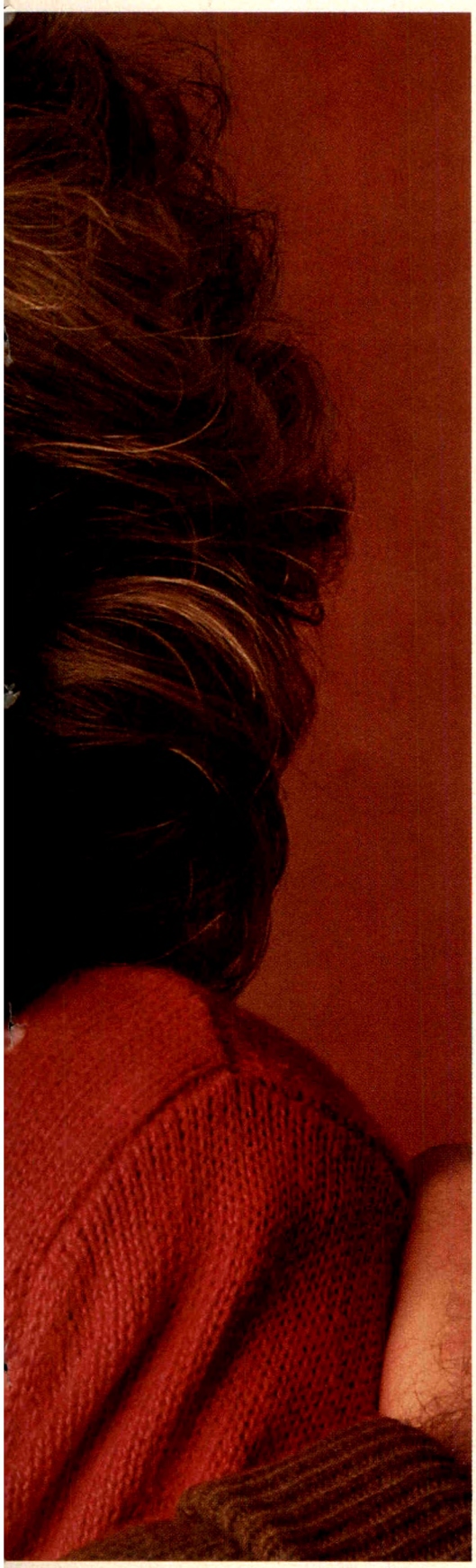
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Note: Estrogens have been reported to increase the risk of endometrial carcinoma in postmenopausal women.

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Please see brief summary of prescribing information on last page of this advertisement.

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PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.
There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risks, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy: changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

Reference: 1. Weiss NS, Ure CL, Ballard JH, et al: Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. *N Engl J Med* 1980;303:1195-1198.

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Manuscript submission. Manuscripts should be submitted to one of the two Editors in Chief as follows:

1. **Dr. Zuspan**—manuscripts from the eastern and southeastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. J. Peter Van Dorsten, P.O. Box 34, MCV Station, Richmond, VA 23298.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

Author's suggested reviewers. When authors submit their manuscripts, they must provide the names and addresses of at least three reviewers for consideration by the Editors.

Copyright statement. The specified copyright statement that follows the Information for Authors in each issue of the JOURNAL must be completed, signed by all authors, and submitted with the manuscript. If not completed in full, it will be returned to the author for completion. The copyright statement and checklist form may be torn from the JOURNAL or photocopied for submission.

Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. *If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.*

Previous publication. If a report by the same author(s) or some of the same authors has been previously published in any medium that deals in any respect whatsoever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article(s) or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. Two copies of a new manuscript being prepared for possible publication also must be submitted. The author(s) should inform the Editor of the circumstances, similarities, and differences of the reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and nonhuman experimentation. It is assumed by the Editors that manuscripts emanating from a particular institution are submitted with the approval of the

requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the *Material and Methods* section of the submitted manuscript. Reports of experiments on nonhuman animals or other species must *state in the* Material and Methods *section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed. The type of nonhuman animals or other species used in an investigation must be named in the title, abstract, key words, and *Material and Methods* section of the manuscript.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. *In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.*

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

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General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of black-and-white glossy prints of illustrations are required. See "Color figures" and "Computer-generated figures" for special requirements. Manuscripts must be submitted in English.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, figure legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable, but honors degrees are not permitted); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, business and home telephone numbers, and fax number of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, business and home telephone numbers, and fax number of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. *At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).*

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page including key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Below the abstract, list 3 to 5 *key words or short phrases* for indexing purposes.

A structured abstract is required for certain types of articles and a *standard abstract format* is required for other types of articles; therefore the author(s) should select and prepare the appropriate abstract to be used in the submitted manuscript. This applies to both independently submitted and society manuscripts.

Structured abstract. A structured abstract, limited to 150 words, is to be used for regular articles, society articles, and Current Investigation articles; excluded are Clinical Opinion, Current Development, case report, and brief communication articles. The structured abstract is to contain the following major headings: *Objective(s); Study Design; Results;* and *Conclusion(s).* The *Objective(s)* reflects the purpose of the study, that is, the hypothesis that is being tested. The *Study Design* should include the setting for the study, the subjects (number and type), the treatment or intervention, and the type of statistical analysis. The *Results* include the outcome of the study and statistical significance if appropriate. The *Conclusion(s)* states the significance of the results.

Standard abstract. The standard abstract format is required for Clinical Opinion, Current Development, case report, and brief communication articles. The standard abstracts for Clinical Opinion and Current Development articles are to be 50 to 150 words and standard abstracts for case reports and brief communications are limited to 50 words. Even though the formal names of these four types of articles are not used, an author should determine his/her type of manuscript and prepare the appropriate standard abstract, regardless of whether it is to be submitted independently or as a part of a society's transactions.

Text. Do not hesitate to write your manuscript in the first person and active voice if they are more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through *Comment* section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

Council of Biology Editors Style Manual or the AMA's *Manual of Style.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify *Material and Methods*, *Results*, and *Comment*. Authors may wish to summarize their findings in a short paragraph at the end of the *Comment* section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to repeat the study) the plan, the patients, experimental animals or other species, material, and controls, the methods and procedures utilized, and the statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the *Comment* section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the *Results* section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number (25) is allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (N Engl J Med 1991;324:424-8). Journal titles should conform to abbreviations used in *Cumulated Index Medicus.*

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Dunnett C, Fischermann E, Quilligan EJ. Vaginal delivery following cesarean section: use of oxytocin augmentation and epidural anesthesia with internal tocodynamic and internal fetal monitoring. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: Ledger WJ. Dystocia and prolonged labor. In: Wilson JR, Carrington ER, eds. Obstetrics and gynecology. St. Louis: CV Mosby, 1987 vol 8:474-93.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. *This signed permission must accompany the manuscript when it is submitted to the Editor.* Published abstracts can be used as numbered references; however, reference to the complete published article is preferred.

Figures. The term *figure* includes all types of illustrations such as graphs, diagrams, photographs, flow charts, and line drawings. A reasonable number of figures will be reproduced without charge, but special arrangements must be made with the Editors for color figures at an additional charge to the author. The quoted charge is per page of color, and as many as eight color figures can be included on one page if the color balance is appropriate.

Figures must be cited consecutively in the text in Arabic numerals and identified with a gummed label on the back of the figure with author(s) name(s), title of article, figure number, and top marked clearly. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Do not use paper clips or mar the surface of figures in any way. Figures will be returned only on request by the author.

For *black-and-white figures,* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy prints with good black-and-white contrast.

For *color figures* submit original transparencies and two sets of unmounted prints on glossy (smooth surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The top, first author's last name, and figure number must be indicated on the front of each transparency and the back of each print.

Computer-generated figures must be legible and clearly printed in jet black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend should not appear on the figure. On the back of each figure the name of the first author and the figure number should be given and the top indicated. Three original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color figures* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color figures.The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Legends to figures. Legends for all figures must be typed together in numeric order double-spaced on one sheet of paper (or more if necessary) separate from the text of the manuscript. This page must be numbered in sequence after the references. Titles should be included in the legend, not on the print. Original magnifications should be provided. If a figure has been taken from copyrighted material, the legend must give full credit to the original source.

Tables. Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Titles should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. If a table or any part thereof has been taken from copyrighted material, a footnote to the table must give full credit to the original source. Arrangements must be made with the Editors for elaborate tables because of space limitations.

Permissions. Direct quotations, tables, or figures that have appeared in copyrighted material must be accompanied by written permission for their use from *the copyright owner and original author* along with complete reference information. Photographs of identifiable persons must be accompanied by signed releases. If not, all recognizable features must be masked.

Requirements for certain types of manuscripts

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include *standard abstract format*, of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article. The abstract *must* follow the *structured format.*

Clinical Opinion. Limit of 3000 words. Include *standard abstract format* of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include *standard abstract format* of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Letters to the Editors. *Letters to the Editors* must be submitted in duplicate, typed double-spaced, and the text of the manuscript must be limited to a maximum of 400 words (excluding references, name[s] and address[es] of the signer[s], and the phrase "To the Editors"). The submitted *Letter to the Editors* must be accompanied by a signed copyright statement. If there are two or more signers of the *Letter to the Editors,* one of the authors may sign the copyright statement but must add to the statement the following: "I sign for and accept responsibility for releasing this letter on behalf of any and all cosigners." These requirements apply to all types of *Letters to the Editors.* Letters should be sent to Dr. Zuspan.

Two types of letters will be considered for publication. (1) A *Letter to the Editors* commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a *Letter to the Editors* will be considered for publication.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Zuspan. No books will be returned.

Reprints

The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the Publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (1-800-325-4177, ext. 4306, or 314-453-4306).

CHECKLIST AND COPYRIGHT STATEMENT

Both the checklist and the copyright statement forms **must be completed** and sent with each submitted manuscript. If **not** completed, these forms will be returned to the author for completion.

These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

—The original and two copies of the manuscript are submitted.
—The completed checklist is enclosed. The assignment of copyright signed by **all** authors accompanies the manuscript.
—Human experimentation has been approved by the local institution as stated in the *Material and Methods* section.
—Guidelines for the care and use of *nonhuman* animals or *other species* approved by the institution have been followed as indicated in the *Material and Methods* section. *The species is named in the title, abstract, key words, and* Material and Methods *section.*
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed in English double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
—All pages are numbered in the following order: title page, condensation, structured or standard abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
—Signed, written permission from both the copyright holder and the original author for the use of tables, figures, or quotations previously published and their complete references are enclosed with the manuscript.
—Signed, written permission for the use of quotations of personal communications and unpublished data has been obtained from the person(s) being quoted and is enclosed.

Authorship

—In the covering letter that accompanies the submitted manuscript I/we have confirmed that all authors fulfilled both conditions required for authorship.

Conflict of interest

—In the covering letter that accompanies the submitted manuscript the commercial association of the author or of any coauthors that might pose a conflict of interest is described.

Previous publications

—Enclosed with the submitted manuscript are two reprints each of articles the author and/or some of the coauthors have previously published, have submitted for possible publication, or have in manuscript form dealing with same patients, same animals, same laboratory experiment, or same data, in part or in full, as those reported in the submitted manuscript. Further explanation is provided in the covering letter that accompanies the submitted manuscript.
—Similarities, differences and explanation are provided in the covering letter that accompanies the submitted manuscript.

Reviewers

—Names and addresses of three suggested reviewers are enclosed.

Title page

—These elements are given in the following sequence, and are typed double-spaced.
—Title.
—Author(s) name(s) and highest academic degree(s) are shown.
—City(ies), state(s), and country other than United States in which the study was conducted are given.
—The name(s) of the institution(s) section(s), division(s), department(s) in which the study was performed is provided and the institutional affiliation(s) of the author(s) at the time of the study is indicated.
—Acknowledgment of financial support is cited.
—Name, address, business and home telephone numbers, and fax number of author to whom requests for reprints are to be sent are included.
—If reprints will not be available, this has been stated on the title page.
—If the corresponding author is different from the author to whom reprint requests are to be sent, his/her name, address, business and home telephone numbers, and fax number have been added.
—At the bottom of the page, a running head not exceeding 52 characters (including word spaces) has been typed.

Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract and key words or short phrases

—The abstract (structured or standard format) is typed double-spaced with required margins on page 3 headed by the title and author(s) name(s). Beneath the abstract 3 to 5 key words or short phrases are typed.
—A *structured abstract,* with 150 words or less, is submitted as required for regular articles, society regular-type articles, and Current Investigation articles. The abstract contains the four required major headings: Objective(s), Study Design, Results, and Conclusion(s), each with a brief adequate presentation.
—A *standard abstract* is submitted as required for Clinical Opinion and Current Development articles with 50 to 150 words and for case reports and brief communications articles with a maximum of 50 words, be they independent or society articles.

References

—Are typed double-spaced.

—Are numbered consecutively in the order they are cited in the text.

—The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors have been followed.

—Personal communications and unpublished observations are not used as numbered references but are mentioned in the text with the written approval of the person being quoted. The signed approval is enclosed.

Figures

—Each is numbered with an Arabic numeral and cited in numeric sequence in the text.

—Three sets of unmounted glossy prints for *black-and-white* figures are submitted, properly numbered, labeled, and *top indicated* on the back of each print.

—Three sets of original computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back and *top indicated* are submitted. None are dot matrix or photographic prints.

—Original transparencies of *color photographs* appropriately numbered and identified and *top indicated* are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

Figure legends

—Are provided for each figure.

—Are numbered and typed together in numeric order on one sheet of paper (more if necessary). The page is numbered in sequence after the References page(s).

Tables

—Each table headed by a title and numbered in Roman numerals is typed double-spaced on a separate page.

—Tables are cited in numeric sequence in the text.

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

COPYRIGHT STATEMENT

Must be signed by ALL authors

"The undersigned author(s) transfers all copyright ownership of the manuscript entitled

to Mosby–Year Book, Inc., in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author."

The responsible author(s) must be named_____ _____
(Type name) Signature

Each author's name must be typed underneath the signature.

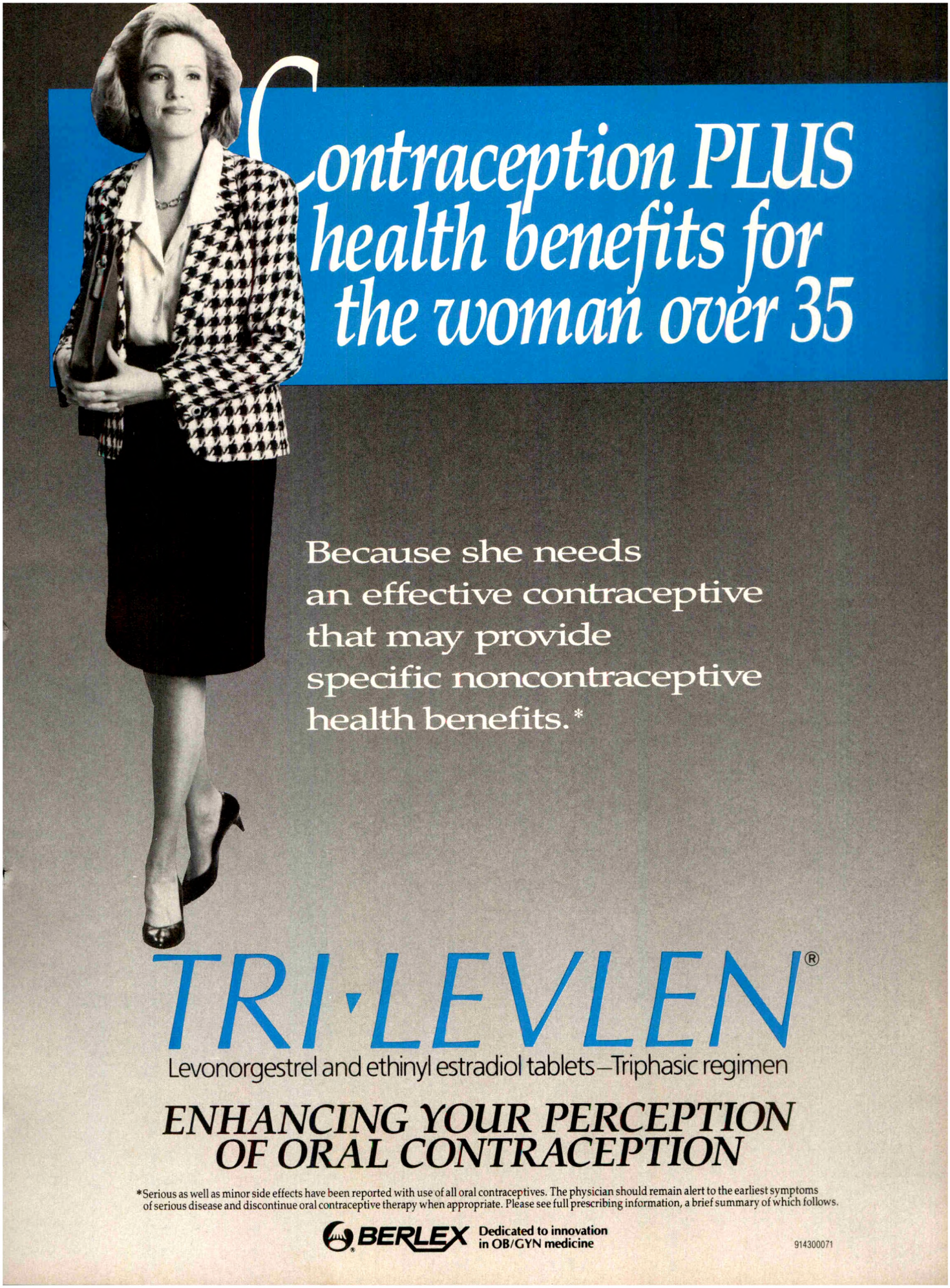
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the woman over 35
Because she needs
an effective contraceptive
that may provide
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health benefits.*
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*Serious as well as minor side effects have been reported with use of all oral contraceptives. The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.
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TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen

TRI-LEVLEN® 28 TRI-LEVLEN® 21

BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or conort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's—but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalias and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minima. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

For full details on dosage and administration see prescribing information in package insert. 60627-0 5/90

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EDITORIAL

Summary of analysis of published articles

September 1, 1990, through August 31, 1991

Each year a retrospective analysis of the articles published in the JOURNAL is presented to the readers and also to the Editorial Board and the Advisory Committee on Policy. Over the past twenty-two years we have compiled data on the classification, category, and type of articles accepted and published. A comparison of the data for the last two years is presented in Table I. A comparison of publication time in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY of regular articles (excluding society articles) for 1986-1987 through 1990-1991 is shown graphically in Fig. 1, on page 774.* The pie charts on page 774 show the articles and page utilization for 1990-1991 by classification (Fig. 2A) and category (Fig. 2B).

*Publication time of regular articles from time of acceptance to publication: 1986-1987, 4.9 months; 1987-1988, 5.2 months; 1988-1989, 5.6 months; 1989-1990, 5.1 months; and 1990-1991, 5.1 months.

Table I. Comparison of data on classification, category, and type of articles published in 1989-1990 and 1990-1991

	1990-1991			*1989-1990*		
	No. of articles	*Pages*	*Average length per article*	*No. of articles*	*Pages*	*Average length per article*
Classification of articles:						
Clinical	461	2439		506	2407	
Basic Science	87	543		130	731	
Total	**548**	**2982**		**636**	**3138**	
Category of articles:						
Obstetrics	197	1048		253	1240	
Gynecology	157	881		151	770	
Fetus, Placenta, Newborn	187	991		224	1091	
Education	7	62		8	37	
Total	**548**	**2982**		**636**	**3138**	
Composition of the JOURNAL based on the type of article:						
Regular	262	1474	5.6	331	1757	5.3
Society	186	1154	6.2	175	998	5.7
Brief Communication/Case Report	72	149	2.1	99	199	2.0
Current Investigation	0	0	—	2	15	7.5
Current Development	10	98	9.8	7	71	10.1
Clinical Opinion	18	89	4.9	22	98	4.5
Total	**548**	**2982**	**5.4**	**636**	**3138**	

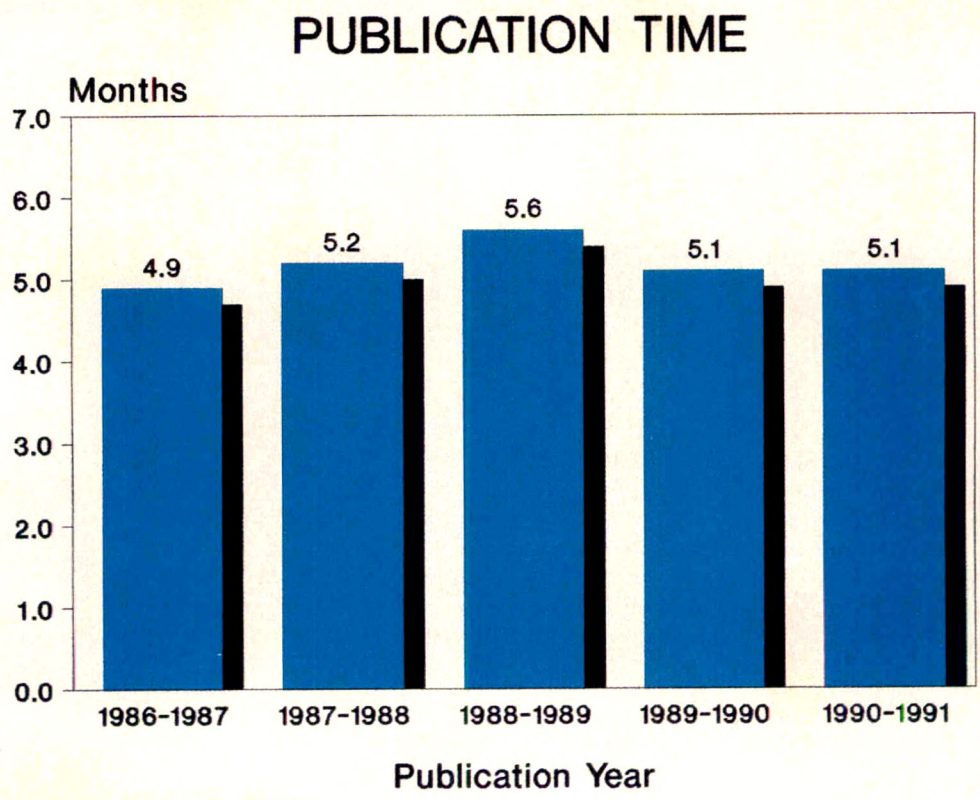


Fig. 1. Five-year comparison of publication time of regular articles (excluding society articles) in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

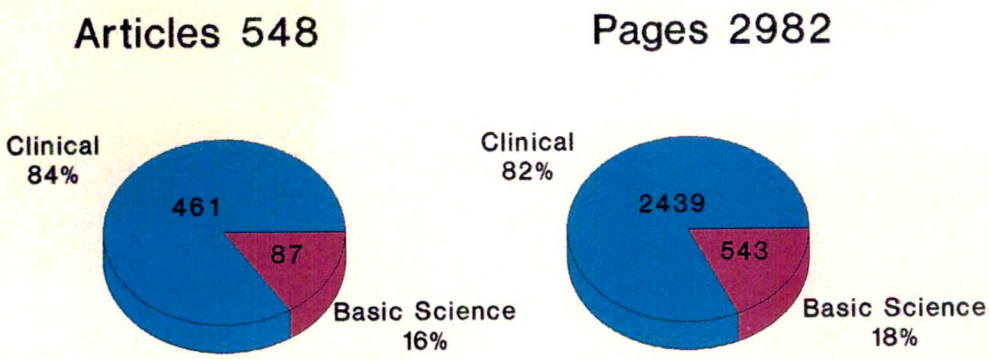


Fig. 2A. Articles and page utilization for 1990-1991 by classification.

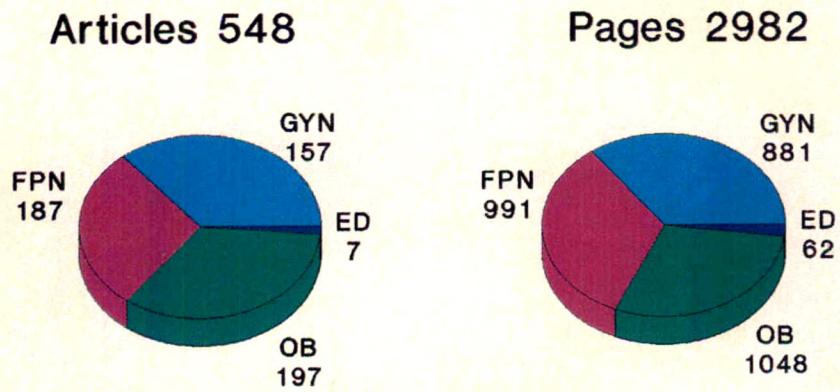


Fig. 2B. Articles and page utilization for 1990-1991 by category.

Embryoscopy: A closer look at first-trimester diagnosis and treatment

E. Albert Reece, MD, Siegfried Rotmensch, MD, John Whetham, MD, Mark Cullen, MD, and John C. Hobbins, MD

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Advancing technology has made the fetus and its environment even more accessible to prenatal diagnosis and treatment. The current approach to prenatal diagnosis relies mainly on the use of high-resolution ultrasonography. However, as attempts are made to conduct antenatal diagnoses earlier in gestation, the limits of ultrasonography are approached. Embryoscopy allows for direct visualization of the first-trimester fetus with a fiberoptic endoscope. A customized side channel enables the operator to pass a variety of diagnostic tools and gain access into the fetal circulation. The further development and refinement of this technology are expected to change early prenatal diagnosis and treatment considerably. The potential contribution of this technique to perinatal medicine is readily apparent when it is placed in historic context. Undoubtedly, many ethical, legal, and regulatory questions will have to be addressed before the full potential of embryoscopy is realized. The responsibility for the judicious use of this powerful technology for prenatal intervention will have to be shared by the scientific community and a well-informed public. (Am J Obstet Gynecol 1992;166:775-80.)

Key words: Prenatal diagnosis, embryoscopy, perinatal medicine

Throughout history, the development of the human fetus has been considered a mystery, hidden within the walls of the uterus. The fetus and its compartment were considered off limits and regarded as a sacred enigma. Pregnancy mysteriously eventuated in either a normal infant or an anomalous one.

Recently, a fundamental transition toward a different concept has occurred. Advancing technology has rendered the fetus and its environment accessible to the "outside" world and therefore to diagnosis and therapy. Among the latest of such technologies is embryoscopy, which utilizes high-resolution fiberoptic equipment for direct visualization of the embryo-fetus. The importance of embryoscopy to perinatal medicine and its potential contributions to the concept of fetal therapy are readily apparent when the technique is placed in a historic context.

In the late 1930s, indirect visualization of the fetus by radiographic techniques, with and without contrast enhancement, began to be utilized. The first direct transuterine visualization of the fetus was successfully performed by Westin[1] in 1954 using a 10 mm hysteroscope. In spite of the image quality being rather limited by the insufficient optical system, fetal parts and movements could be identified. Nevertheless, even without optimal delineation of the fetal structure, the invasion of the fetal compartment had begun, and the stage was set for targeted fetal diagnosis and direct in utero therapy.

The advent of amniocentesis for Rh isoimmunization in the 1950s, followed by genetic amniocentesis in the 1960s, was a major milestone in the development of fetal intervention. Donald et al.[2] introduced ultrasonography into obstetrics and gynecology in 1958 and paved the way for the widespread use of an extremely powerful modality of fetal visualization. The static-image ultrasonographic scan enabled us to learn about fetal and uterine morphologic features and resulted in a blossoming of descriptive literature on the fetus. Before long, advancing technology yielded the gray-scale dynamic ultrasonographic image, which added yet another dimension to our understanding. Not only did the real-time scanner improve visualization, but for the first time some aspects of human fetal physiology were observed in vivo. Investigators were fascinated by the "live" observation of fetal body and limb movements,

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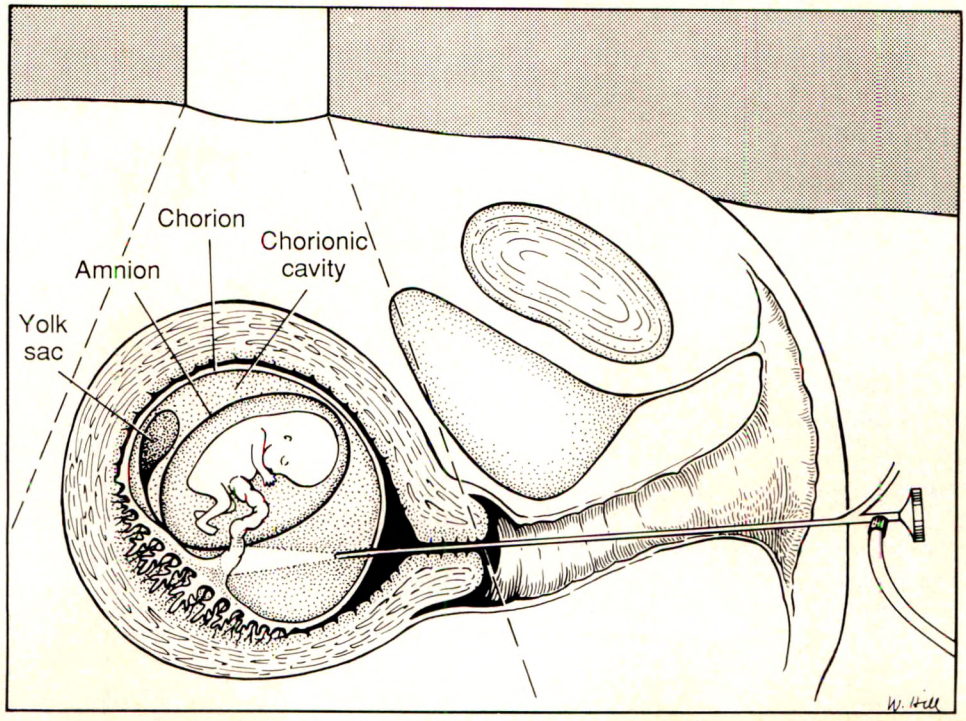


Fig. 1. Schematic diagram of embryoscopy. Sector ultrasound transducer is seen on maternal abdomen as it is used to guide entry of endoscope through cervix and into chorionic cavity. Visualization of entire embryo-fetus is feasible.

breathing movements, bladder filling and emptying, heart motion, swallowing, and gastric filling dynamics. Circadian rhythms and periodicity of fetal physiologic functions were discovered and correlated with maternal physiologic status.[3]

Prenatal diagnosis of congenital anomalies improved exponentially with the advanced resolution of modern ultrasonography equipment.[4] In conjunction with electronic fetal heart rate monitoring, a rapidly evolving concept of fetal well-being emerged to have a significant impact on the practice of obstetrics. The interest of our specialty shifted its focus from the delivery process and associated surgical skills to the quest for understanding fetal biology. It became clear that fetal pathologic conditions could not be interpreted correctly if normal fetal biologic characteristics remained insufficiently understood.

The missing link for the achievement of this goal was access to the fetal circulation. Most physiologic and biochemical processes in the fetus were expected to result in alterations of metabolic products in the fetal blood circulation, similar to those of an adult. Boenisch and Saling[4a] translated this concept into the technique of intrapartum determination of fetal capillary scalp blood pH for detection of fetal distress. This technique was an important step toward an understanding of fetal acid-base status; however, it remained limited to parturition.

Initial attempts at gaining access to the fetal circulation during fetal development focused on ultrasonography-guided aspiration of fetal blood from the placenta. This approach was hampered by significant difficulties in obtaining pure fetal blood specimens and by relatively low success rates. In the early 1970s, Valenti[5] and Scrimgeour[6] reported the use of 6 and 2.2 mm endoscopes, respectively, for examination of the human fetus. Their techniques involved exposing the uterus by laparotomy and inserting the instruments through myometrial incisions. The widespread diagnostic and therapeutic use of fetoscopy in ongoing pregnancies became feasible when Hobbins and Mahoney[7] developed the Dyonics needlescope instrument, which used an ultrasonographically guided percutaneous transabdominal technique with the patient under local anesthesia. Direct fetal visualization allowed for the diagnosis of external structural anomalies, aspiration of fetal blood, and skin, liver, and fetal tumor biopsies, as well as for intravascular infusion of blood and blood products.[8, 9] The foundation for fetal medicine as a subspecialty in its own right had been laid.

Major drawbacks of fetoscopy, however, were related to a fetal loss rate of 4% to 8%, as well as to inconsistent success in obtaining fetal blood samples devoid of amniotic fluid contamination[10]; risk-versus-benefit considerations somewhat limited the use of fetoscopy. Addi-



Fig. 2. As endoscope is passed transcervically into exocoelomic space, clear, wavy amnion can be seen, through which undersurface of "placenta" can be visualized.

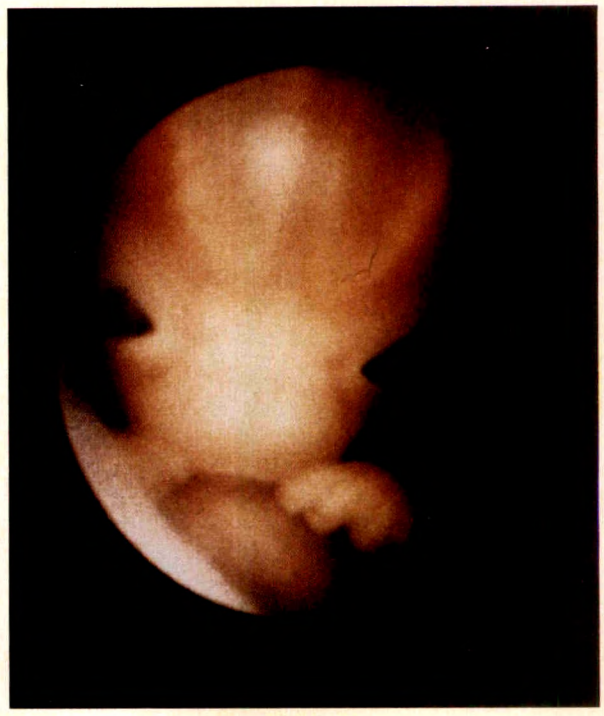


Fig. 3. Direct visualization of fetus at 9 weeks (menstrual). Head is seen flexed on its chest with prominent forehead, normal separated eyes, and fused lips. Fingers at this age are only slightly webbed.

tionally, fetoscopy could not be used when the amniotic fluid was discolored and was less efficacious in advanced pregnancies.

With the dramatic improvement in ultrasonography technology, Daffos et al.[11] were the first to substitute direct vision for ultrasonographic guidance of a spinal needle for percutaneous umbilical blood sampling. This technique quickly replaced fetoscopy for many diagnostic and therapeutic indications because of its safety, simplicity, and ability to obtain 100% pure fetal blood. Consequently, our understanding of fetal infectious disease, fetal metabolism, and transplacental pharmacologic characteristics was greatly enhanced, and important work on the pathophysiologic parameters of intrauterine growth retardation and fetuses of diabetic mothers continues.

All of the above-described diagnostic and therapeutic techniques are limited to use during the second and third trimesters; the first-trimester embryo and fetus have so far received only some diagnostic and relatively little therapeutic attention. Of course, chorionic villus sampling (CVS) can be performed as early as 6 weeks' gestation and provides extremely valuable genetic information. Furthermore, the utility of CVS has recently been enhanced by powerful techniques of deoxyribonucleic acid (DNA) analysis and our growing knowledge of DNA sequence. However, the diagnostic capability of CVS is limited by the degree to which trophoblastic and embryonic tissue is genetically congruent. Additionally, non–genetically determined structural anomalies are beyond the diagnostic scope of CVS. First-trimester ultrasonography, particularly transvaginal scanning, can diagnose major anomalies relatively early.[12] It appears, however, that the limits of sound wave resolution, with currently used frequencies, have been rapidly approached, particularly when it comes to minor malformations. None of these techniques can be used for treatment of the embryo.

Embryoscopy

Embryoscopy is a recently introduced technique that consists of transcervical fiberoptic endoscopy of the first-trimester embryo-fetus. It can be performed as early as 5 weeks' menstrual age. A side channel, customized for our instrument, allows for the passage of an aspiration needle for access to the embryonic blood circulation. Currently, this is the only instrument that allows direct visualization of the human embryo in vivo and access to its circulation. Multiple diagnoses of embryonic malformations have been made by embryoscopy; however, the diagnostic and therapeutic potential is just beginning to unfold.

Technique

The technical aspects of the procedure have been described elsewhere.[13, 14] In brief, embryoscopy utilizes

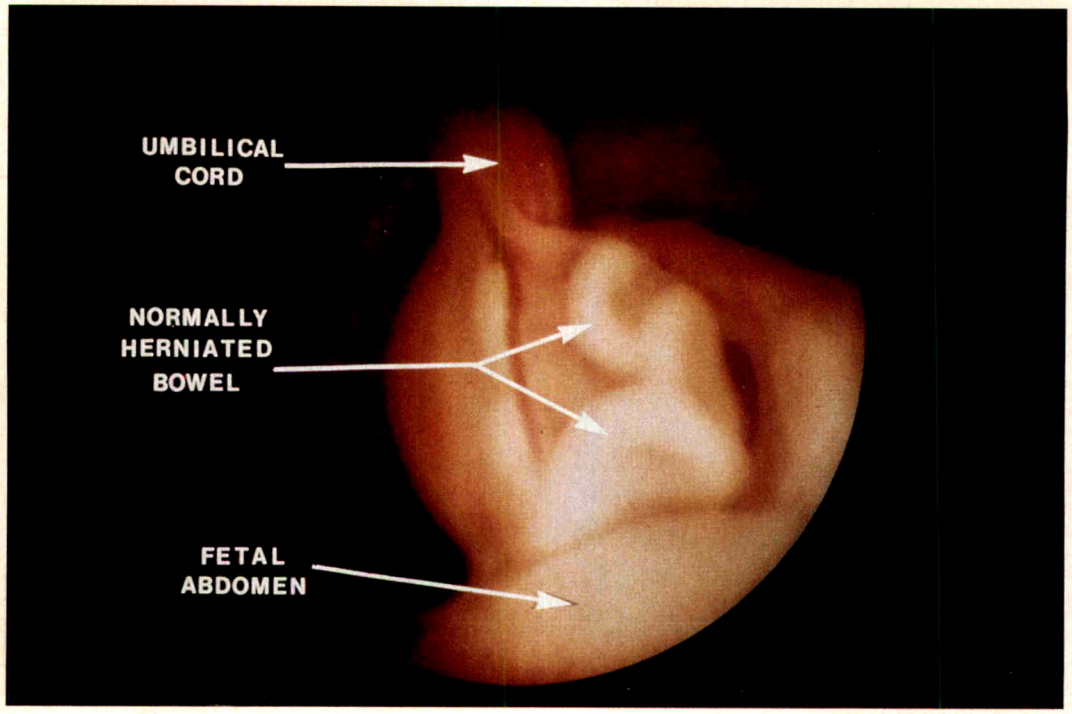


Fig. 4. Fetus with physiologic hernia at 9 weeks' gestation. Bowel can be seen herniating in base of umbilical cord. After 11 weeks' gestation failure of bowel contents to return leads to diagnosis of omphalocele.

a rigid fiberoptic endoscope, 30 cm in length, with diameters from 2.0 to 3.5 mm and a 0- or 30-degree angle lens. With ultrasonographic guidance, the endoscope is passed transcervically into the extracoelomic cavity (chorionic cavity) without disturbing the amnion (Figs. 1 and 2). A complete examination of the embryo or fetus includes visualization of the head, face, dorsal and ventral walls, limbs, umbilical cord, and yolk sac. The average length of time for this procedure is 5 minutes, and the success rate for visualization of the fetus is above 95%. Dumez[15] has reported a similar success rate. He has also performed embryoscopy, undertaken to rule out head and limb anomalies, in >50 continuing pregnancies. In his early attempts two pregnancies were lost, but in subsequent work no adverse outcome was noted. In our experience the inadvertent perforation of the amnion occurred most frequently at or beyond 12 weeks' gestation, when the chorion and amnion were fused. It should be emphasized that overall relatively few ongoing pregnancies have so far been investigated, but this is expected to change with further improvement in the technique.

Applications

Embryoscopy can be performed as early as three conceptual weeks (5 weeks' menstrual age) and continued throughout the first trimester. The procedure is therefore particularly suitable for the documentation of developmental events of the embryo and fetus and for the diagnosis or reconfirmation of malformations.[16]

The fetal face and head can be examined from six menstrual weeks of gestation on (Fig. 3). Gross malformations such as anencephaly and acrania are therefore likely to be diagnosed by embryoscopy. Hypotelorism, hypertelorism, and the absence of one or both eyes are other anomalies that should be easily visualized by direct examination of the fetus. The same holds true for ventral wall defects (Fig. 4). We have diagnosed a case of omphalocele at 10 weeks' gestation by embryoscopy. Limb deformities are particularly well visualized. We have also diagnosed polydactyly at 11 menstrual weeks of gestation, and it appears feasible to diagnose many other limb malformations as well, because of the clear delineation of limb anatomy on direct visualization. Thus embryoscopy can serve as a primary diagnostic tool for congenital anomalies in the first trimester or for confirmation of ultrasonographically suspected anomalies.[16]

Furthermore, from a conceptual point of view, embryoscopy will be of increasing importance to general human embryology. Much of our knowledge of early human development is based on the investigation of human abortuses or analogies drawn from animal research. It is conceivable, however, that the very same abnormality that causes a miscarriage could affect fetal growth and development as well, thereby somewhat

limiting the validity of the former approach. On the other hand, stages of embryonic development in animals are not necessarily representative of human development, thereby limiting the validity of the latter approach. Embryoscopy visualizes the human embryo in vivo, unaffected by any abnormality of the uterine or hormonal environment and representative of the intrinsic capability of a given fetus for early development.

In this context a major question awaiting elucidation is the unclear role of the yolk sac in early human development. The yolk sac provides blood cell precursors, gonadocytes, and epithelia of the digestive and respiratory tracts and has been demonstrated to be structurally altered when exposed to high glucose concentrations in embryo-culture experiments. It has been suggested therefore that the yolk sac could play an essential role in the pathogenesis of congenital anomalies among fetuses of diabetic mothers.[17-20] Direct observation of the yolk sac by embryoscopy, as well as aspiration of its contents for laboratory analysis, could enhance our understanding of human malformations considerably.

Last, first-trimester diagnosis and treatment of genetic diseases by embryoscopy are very intriguing. Embryonic organ-tissue biopsy and/or embryonic blood sampling certainly appear to be within reach. Rapid progress has also been made in recent years toward the concept of human cell and gene therapy. Even though human gene therapy has not yet been approved for clinical trials, we are at the threshold of an era in which this new therapeutic approach will probably be used in the treatment of currently incurable diseases. Obviously, the in utero application of this technology would be limited to serious genetic diseases that produce irreversible damage by the time of birth. At present, the only human tissue that can be used effectively for gene or cell transfer is bone marrow. It is expected, however, that more will be learned in the future about how to package the DNA and to make it tissue specific. It would then be feasible to use the intravenous route for injection of genetic material and for access to the embryo and its circulatory system.

The potential feasibility of the above approach has been tested by Kantoff et al.[21] They demonstrated expression of the human adenosine deaminase (ADA) gene in the blood cells of irradiated monkeys that were reinfused with their own bone marrow cells after the cells had been treated in vitro with an ADA retroviral vector. The first attempts at postnatal human therapy of ADA deficiency are at advanced stages. Other genetic diseases, particularly those that affect the brain (e.g. Lesch-Nyhan disease), will require in utero intervention to be effective. Because in utero transplantation of donor tissue might become necessary to achieve this goal, embryoscopy could be an invaluable access tool to the early embryo-fetus, which would allow for graft acceptance by a "naive" immune system.

Embryoscopy therefore is expected to have a major impact on fetal medicine in the years to come. Prenatal diagnosis and therapy will change considerably, with more emphasis placed on the first-trimester fetus. Undoubtedly, many ethical, legal, and regulatory questions will have to be addressed before the full potential of embryoscopy is realized. The responsibility for the judicious use of this powerful technology for prenatal intervention will have to be shared by the scientific community and a well-informed public.

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Maternal and fetal outcome after breast cancer in pregnancy

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We compared 118 women with breast cancer (119 pregnancies) with 269 nonpregnant control subjects matched on important prognostic factors. The distribution of breast cancer stages among the 118 pregnant women was compared with that among 5115 cases of breast cancer in women of reproductive age. Fetal outcome was compared with that of a control group matched for maternal age. Women having breast cancer in pregnancy were 2.5 times more likely to have metastatic disease (95% confidence interval 1.1 to 5.3) and had a significantly lower chance of having stage I disease ($p = 0.015$). Survival of pregnant women did not differ from that of the controls. Birth weights of babies born to women with breast cancer were significantly lower than those of control babies after gestational age was adjusted for (3010 ± 787 vs 3451 ± 515 gm, $p = 0.016$). The two stillbirths in 85 pregnancies that continued to term (2.4%) was not statistically different from the 1.1% rate for Ontario. We analyzed all 223 births occurring in women who had any form of cancer in the same hospital during the same 30 years. There were 10 stillbirths among these 223 cases (4.4%), significantly more than expected in Ontario ($p < 0.0005$; relative risk of 4.23 with 95% confidence interval 2.0 to 7.8). Our data suggest that pregnant women are at a higher risk of presenting with advanced disease because pregnancy impedes early cancer detection. (AM J OBSTET GYNECOL 1992;166:781-7.)

Key words: Pregnancy, breast cancer, fetus, chemotherapy, survival

Cancer occurring during pregnancy poses a very difficult challenge to the woman, her family and her physicians, because cancer therapy may be detrimental to the unborn baby. Equally difficult, postponement of therapy may theoretically decrease her chances of survival. Traditionally, cancer during pregnancy was believed to be associated with poor prognosis and an increased risk of harm to the fetus.[1-3] This impression may have led to unnecessary fear on the part of the patient and her physician; conversely, some recent studies have indicated that the pessimistic view is not based on objective data.[4-7]

Breast cancer is the most common tumor in women of reproductive age; 3% of breast cancer occurs in pregnancy.[8,9] Because of the relative rarity of breast cancer in pregnancy, there is a paucity of information on the effects of pregnancy on the course of breast cancer and the effect of the disease and its therapy on pregnancy outcome.

Using a large data base accumulated for >30 years in an oncologic hospital in Toronto, we undertook a historic cohort study to assess the effects of pregnancy on the diagnosis and course of breast cancer and the impact of the disease and its treatment on fetal outcome.

Material and methods

Princess Margaret Hospital is an oncologic hospital in Toronto serving the province of Ontario. It maintains a computerized data base of patient records dating back to 1958; this data base was used to identify the patients for the study. Patients were recruited from 1958 to 1987. The Motherisk Program, based at the Hospital for Sick Children in Toronto, is an antenatal counseling service for women exposed to drugs, chemicals, and radiation during pregnancy and lactation.

All women with histologically proven breast cancer

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Table I. Staging method

Tumor	*Node*	*Metastasis*	*Stage*
T0	N0	M0	0
T1, T2, T3	N0	M0	I
T1, T2, T3	N1, N2, N3	M0	II
T4	Any	M0	III
Any	Any	M1	IV

in pregnancy registered in the data base between 1958 and 1987 were identified. On the basis of the assumption that cancer would be present for some time before it could be diagnosed, the time frame for inclusion in this analysis selected women who were pregnant within 9 months before or 3 months after first treatment. Because the date of diagnosis was often not recorded in the data base, we used the date of first treatment as the reference date, assuming that there would be minimal delay from the time of diagnosis to treatment (surgical, radiologic, or medical). Extreme cases included in our study were women who were delivered 9 months before first treatment and women who conceived 3 months after the last day of their first treatment.

Potential cases identified by the computer were further screened by examination of their charts to confirm that pregnancy and breast cancer occurred according to the time frame defined and to reject any patients who had an ectopic pregnancy.

To study the potential effects of pregnancy on women's survival, we matched women having breast cancer in pregnancy to nonpregnant women with the tumor. For each case an attempt was made to identify three matching controls in the data base according to these criteria: (1) The control woman could not have been pregnant within 15 months prior to 9 months after first treatment. A 6-month addition on both sides of the time frame ensured that a pregnancy would have no effect on the cancer and that the cancer would have no effect on a pregnancy. (2) Controls had the same stage of breast cancer as the cases at diagnosis. The clinical stage was used for both cases and controls when available, and pathologic evidence was used when the clinical stage was not recorded (Table I). (3) Controls were within 2 years of the ages of cases at diagnosis. (4) Controls had diagnosis or first treatment within two calendar years of the matched case. Type of treatment was not explicitly matched; however, strict hospital protocol dictated that patients of the same age, stage, and calendar year of diagnosis would be given the same treatment. The validity of this assumption was verified in a random sample of 10 pregnant women and 20 controls, which showed identical treatment in 16 (80%) and very similar treatment in the remaining 4 (20%) (minor changes in protocol).

Details of the breast cancer, TNM system staging, and date of diagnosis were recorded for the cases and controls. All cases and controls were rated by the TNM system of 1987.[10] Charts with an ambiguous TNM rating were assessed from the pathology reports and clinical workup. Table I illustrates the staging method.

Dates and types of treatment, including whether treatment was delayed because of the pregnancy, were also obtained. For obstetric information, date of conception, gestational age at diagnosis and first treatment, complications in pregnancy, if any, and pregnancy outcome were recorded.

To compare the distribution of disease stages on diagnosis of breast cancer in pregnancy with that of nonpregnant women of reproductive age (≤47 years) registered in Princess Margaret Hospital, the Princess Margaret Hospital data base was used, in which 3949 cases had an identifiable stage and an additional 1166 cases were identified as clearly not being stage IV (either I, II, or III) for the same calendar years.

For pregnancies that continued to term or resulted in stillbirth, birth records were requested from the delivering hospital. For live births, sex and birth weight of the infant were recorded, as well as gestational age at delivery, type of delivery, Apgar scores, fetal complications, and congenital anomalies. In the case of a cesarean section, the reason for choosing this method was recorded. For intrauterine death, date of diagnosis and autopsy results were collected.

Fetal outcome after maternal breast cancer as compared with that of babies born to women attending the Motherisk clinic during the first trimester after exposure to drugs, chemicals, or radiation during pregnancy. For this analysis each mother with breast cancer who had a live birth was matched with a mother of similar age who was exposed to nonteratogenic drugs or chemicals.

Statistical analysis. Kaplan-Meier[11] estimates were used to produce survival curves, and the Mantel-Haenszel log rank test[12] was used to compare the cause-specific survival of cases and their matched controls. The χ^2 test was used to compare the distribution of tumor stages on diagnosis between the cases and the nonpregnant women of reproductive age having breast cancer and registered in the data base. Fetal outcome values between the study and control groups were compared by two-sided Student *t* test for unpaired results. When birth weight was adjusted for the effect of gestational age, analysis of covariance was applied. The odds ratio was used to estimate the relative risk that a pregnant woman with cancer would have advanced disease[13] and that a pregnant patient with cancer would have a stillbirth. The observed number of stillbirths was assumed to follow a Poisson distribution. Statistical analysis was performed with SAS version 5.1 and Minitab release 6.1.1.

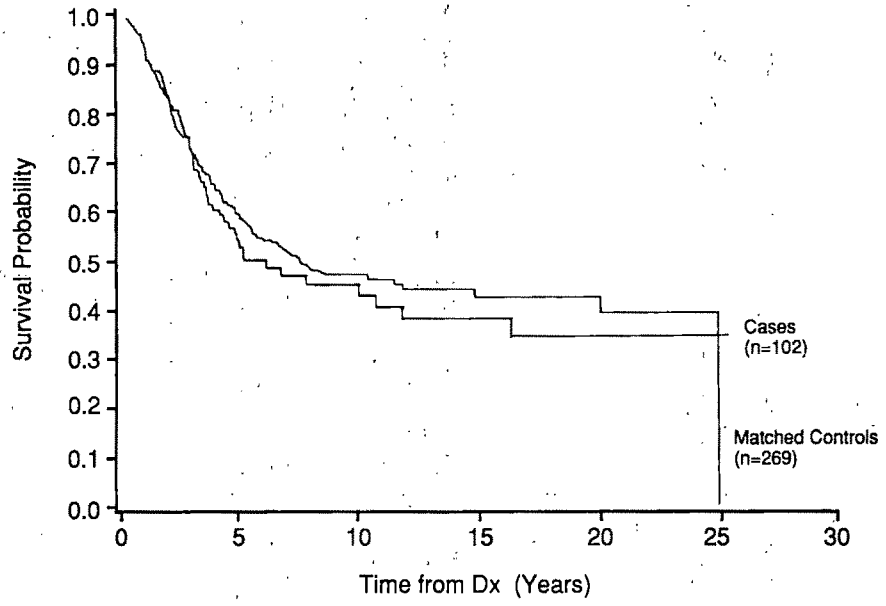


Fig. 1. Kaplan-Meier cause-specific survival curve for breast cancer comparing women who were pregnant and had breast cancer ($n = 102$) against matched nonpregnant controls ($n = 269$), $p = 0.6$.

Results

There were 118 cases of breast cancer and pregnancy identified in the 30 years analyzed in our study. One woman had two pregnancies, both of which fit in the time frame of the study, resulting in a total of 119 pregnancies in 118 women. The mean age of pregnant patients with breast cancer was 32.9 ± 5.13 years at diagnosis. The median age was 33 years (range 23 to 47). Of the 119 pregnancies, 14 women were diagnosed with breast cancer before conception, 42 during pregnancy, and 55 after delivery or termination of the pregnancy. In eight cases when conception occurred relative to diagnosis was not recorded.

Of 118 women, 22 had surgery as first treatment, five received radiation, three received chemotherapy, 45 had surgery and radiation, 20 had surgery and chemotherapy, two received radiation and chemotherapy, and 21 received all three. Of the women with diagnosis before or during the pregnancy ($n = 56$) who received treatment during the pregnancy ($n = 42$), 24 had surgery, five radiation, two chemotherapy, three both surgery and chemotherapy, and eight both surgery and radiation. Of this group of women, there were 16 elective abortions, two miscarriages, and 38 live births. Sixteen women chose to delay the recommended treatment until after delivery or termination (two of whom had surgery and delayed chemotherapy). Only five babies were exposed in utero to cancer chemotherapy, three of them during embryogenesis. These three women chose to terminate the pregnancy.

There were 269 matched controls identified for 102 cases. Three controls per patient could not always be obtained because the matching criteria were selective enough that the data base did not always contain three matches per case. For those women who had matched controls, the distribution of types of treatment between cases and controls was not statistically different ($p > 0.75$). There were 16 cases that did not have matched controls. Six patients were diagnosed with breast cancer in the late 1950s or early 1960s and could not be matched because computer records of that time contained ambiguous staging codes. Seven patients had no matches because the disease could not be properly staged, and the remaining three had no matches in the data base. Review of these 16 charts did not reveal any systematic features that would explain why these patients could not be matched.

A Kaplan-Meier plot reveals that survival did not differ statistically between the pregnant and nonpregnant women ($p = 0.6$, Fig. 1). At the time of the study, 52 of the cases had died of breast cancer, whereas the remaining 50 either were alive or had died of other causes. In the control group 129 patients died of breast cancer.

Fig. 2 compares the subgroup of patients who were diagnosed with breast cancer before conception or during pregnancy ($n = 44$) with their matched controls ($n = 118$), showing virtually identical survival over 15 years ($p = 0.05$).

For women who were diagnosed with breast cancer during pregnancy and maintained the pregnancy until delivery ($n = 32$), five were diagnosed during the first trimester, 11 during the second, and 16 during the third. Fig. 3 shows no statistically significant difference

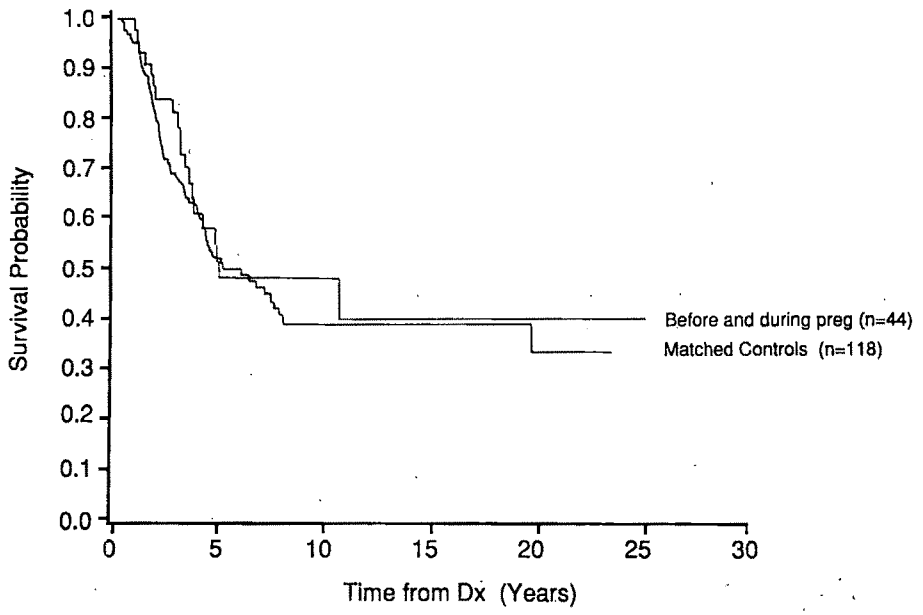


Fig. 2. Kaplan-Meier cause-specific survival curve for breast cancer comparing women whose diagnosis was before or during pregnancy ($n = 44$) against their nonpregnant matched controls ($n = 118$), $p = 0.5$.

in survival among the three trimesters ($p = 0.8$). Fig. 4 compares the survival of women who maintained the pregnancy until delivery ($n = 32$) with that of those whose pregnancy terminated either electively ($n = 9$) or spontaneously ($n = 1$). No statistically significant difference was found ($p = 0.5$). Women who delayed treatment until after delivery did not have a worse prognosis than their matched controls (11 cases and 28 controls, $p = 0.05$); however, because of the small sample sizes in Figs. 3 and 4, the power is low.

Among 111 cases with available staging, 51 (45.9%) had stage I disease at diagnosis, 44 (39.6%) stage II, six (5.4%) stage III, and 10 (9.0%) stage IV. The cause-specific survival by stages is shown in Fig. 5. A statistically significant difference in survival was found among the stages ($p = 0.001$).

Through the computerized data base, we identified 3949 women of reproductive age (≤47 years) in the same time period (1958 through 1987) for whom complete staging was available and an additional 1166 women for whom partial information could confidently rule out stage IV disease.

Of the 3949 women, 2288 (57.9%) were diagnosed with stage I disease, 1293 (32.7%) with stage II, and 169 (4.3%) with stage III. There were 199 women diagnosed with stage IV disease out of 5115 eligible cases (3.9%). This distribution is statistically significantly different from that of the pregnant women ($0.025 < p < 0.05$). After analysis by individual stage and adjustment for multiple comparisons (Bonferroni method), a statistically lower number of women diagnosed with stage I disease in the pregnant group was found as compared with the data base ($p = 0.015$). Similarly, after we adjusted for multiple comparisons, there was a statistically higher proportion of women diagnosed with stage IV disease in the pregnant group compared with the data base ($p = 0.013$). The relative risk of having stage IV disease in pregnancy was 2.5 (95% confidence interval 1.1 to 5.3).

Fetal outcome. Of the 119 pregnancies, 22 were terminated by elective abortion and 12 by miscarriage, there were 85 deliveries. Of the 85 deliveries, there were 83 live births and two stillbirths. Twenty-one of the deliveries were by cesarean section; 18 of these were performed to allow initiation of therapy for breast cancer, whereas the remaining three were for non-cancer-related obstetric difficulties. We were able to obtain 62 obstetric records from the delivering hospitals and one autopsy report. The remaining delivery records could not be obtained because some charts did not indicate the delivering hospital or the delivering hospital had destroyed old birth records. In some cases the delivering hospital refused to release confidential documents because of strict hospital guidelines. A summary of the available information regarding fetal outcome is presented in Table II.

There was a statistically lower mean birth weight for babies of mothers with breast cancer when compared with their matched controls ($p = 0.002$). Similarly, they had a statistically shorter mean gestational age ($p = 0.006$) because of a significantly higher proportion of preterm deliveries ($p = 0.003$).

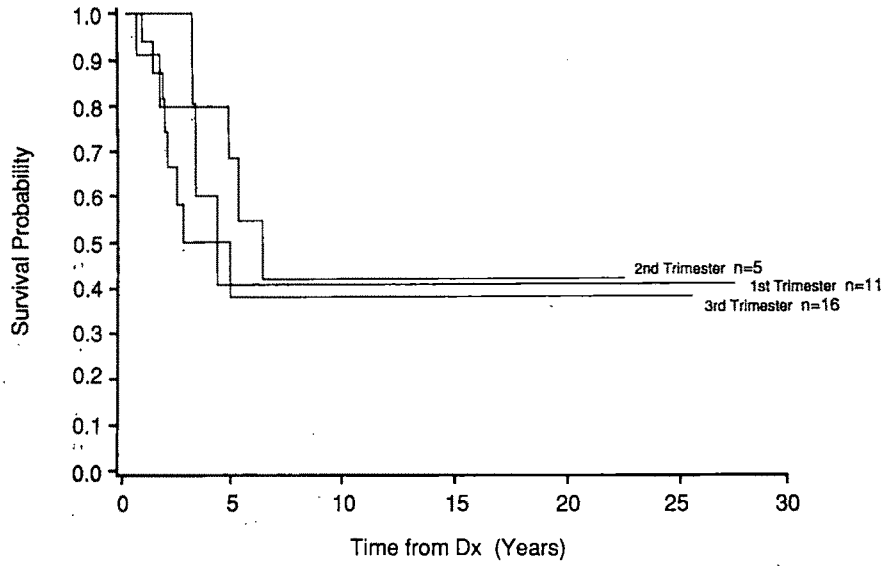


Fig. 3. Kaplan-Meier cause-specific survival curve for breast cancer comparing survival for pregnant women with diagnosis during first (n = 5), second (n = 11), and third (n = 16) trimesters, p = 0.8.

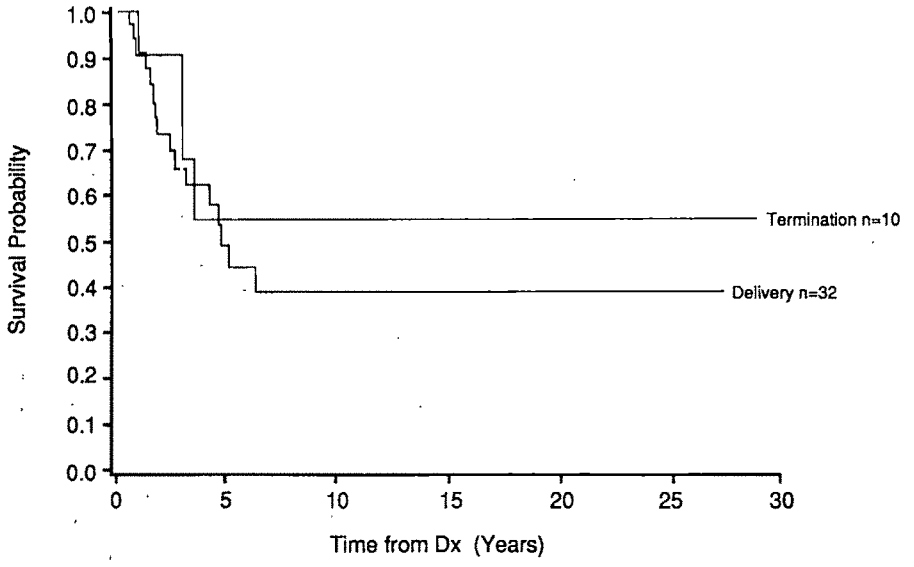


Fig. 4. Kaplan-Meier cause-specific survival curve for breast cancer comparing women who were delivered (n = 32) against those whose pregnancy was terminated (n = 10), p = 0.5.

To assess whether the lower birth weight in babies of cancer patients was due to a greater proportion of premature births or whether the babies were small for gestational age, an adjustment for gestational age was performed by analysis of covariance. This difference was still statistically significant (p = 0.016).

The ratio of stillbirths to live births in our study (2/85, 2.4%) appears larger than the general figure in Ontario (11.1 stillbirths per 1000 total births[14]); however, this difference was not statistically significant. To further verify whether this tendency represents a true risk for stillbirth, we analyzed all 223 births occurring in women who had any form of cancer at Princess Margaret Hospital during the same 30 years. There were 10 stillbirths out of the 223 deliveries, a significantly greater ratio than in the population of Ontario ($p < 0.0005$). This stillbirth rate represents a 4.23 relative risk (95% confidence interval 2.0 to 7.8).

Comment

Because the occurrence of breast cancer during pregnancy is relatively rare (3% of all breast cancers), it is highly unlikely that prospective trials will ever be large enough to assess the effect of breast cancer and its

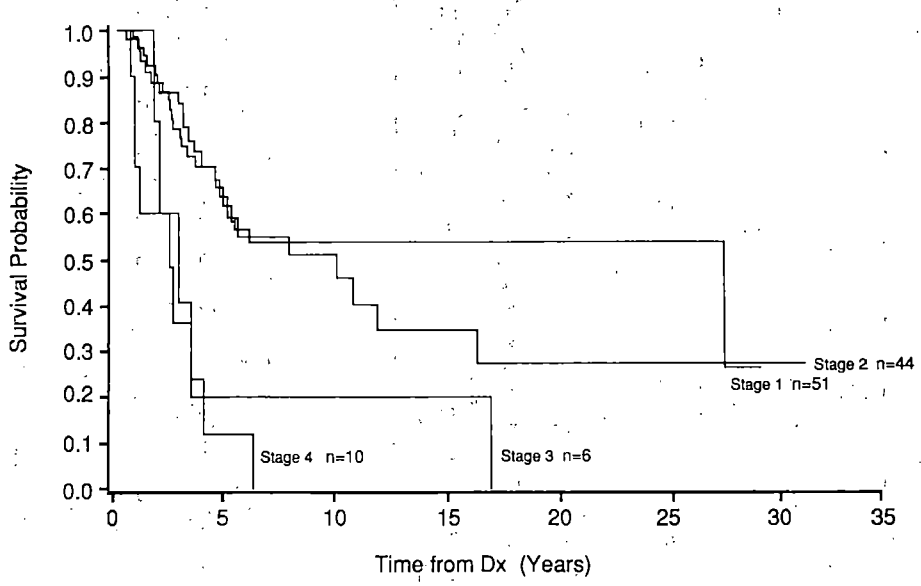


Fig. 5. Kaplan-Meier cause-specific survival curve for breast cancer of pregnant women by stage: stage I *(1)*, $n = 51$; stage II *(2)*, $n = 44$; stage III *(3)*, $n = 6$; stage IV *(4)*, $n = 10$ ($p = 0.001$).

Table II. Comparison of fetal outcome in mothers with breast cancer with that of the matched control group ($N = 73$)

	Total No.	*Study babies*	*Total No.*	*Matched control babies*
Gestational age (wk, mean ± SD)	60	38.3 ± 2.4*	73	39.4 ± 1.8
No. of preterm births (<37 wk)	60	16	73	5
Birth weight (gm, mean ± SD)	44	3010 ± 787†	73	3451 ± 515†
Delivery method				
Spontaneous vaginal		37		59
Cesarean section		20		14
Malformations	73	None	73	None

*$p = 0.006$.
†$p = 0.002$.

treatment on maternal and fetal outcome. To date, most available studies have analyzed the effect of pregnancy by comparing the course of breast cancer in pregnant women with that in a cohort of unmatched controls, thus carrying a risk of bias because of unbalanced prognostic factors.[7, 15, 16] The few studies that used a case-control analysis comprised a small number of patients.[6, 17-19] We found no statistically significant difference in cause-specific survival between pregnant and nonpregnant women matched by age, stage, and year of diagnosis. However, our data reveal that a pregnant woman has a 2.5-fold higher risk of being diagnosed with metastatic breast cancer and a decreased chance of diagnosis of stage I. Because the prognosis of metastatic breast cancer is poor (Fig. 5), this observation means that pregnancy per se poses a risk to women with breast cancer. Similar findings have been suggested by Clark and Reid,[4] Nugent and O'Connell,[7] Ribeiro et al.,[16] and Petrek et al.[20] However, none of these studies used matched controls to substantiate their hypotheses.

Torres and Mickal[21] and Donegan[22] have suggested that during pregnancy the tumor is stimulated by the elevated estrogen levels, implying that the tumors are estrogen receptor positive. However, it has been shown that young women, pregnant or not, usually have estrogen receptor–negative breast cancer.[7] Estrogen receptor–negative tumors have a biologically aggressive course and carry a poor prognosis.[23] Thus it is theoretically conceivable that pregnancy, with its hormonal stimulation, should not affect the course of the disease. This suggestion is supported by the observation that, after they were matched for tumor stage, our pregnant patients had survival identical to that of the nonpregnant controls. Furthermore, termination of pregnancy was not associated with improved survival.

Our data suggest that pregnant women are at a higher risk of presenting with advanced disease because pregnancy impedes early detection. During pregnancy a woman's body undergoes substantial physiologic changes, including enlargement of the breasts, that make it more difficult to notice small lumps that forewarn of cancer.[24] It is probable that many women and possibly their physicians relate findings consistent

with breast cancer to pregnancy-induced engorgement. It has been reported that the average delay from first symptoms to treatment exceeds 5 months.[6]

A pregnant woman may wish to delay her treatment for fear of the effects on the unborn baby. Among the regimens used in the treatment of breast cancer, chemotherapy has by far the highest likelihood of adversely affecting the baby during embryogenesis.[25, 26] The effects of general anesthesia and surgery on the fetus have been investigated by several researchers, and unless complications occur (e.g., shock, hyperthermia), they do not appear to increase fetal risk.[27, 28] Radiotherapy has the potential of causing irreversible harm to the fetus when the fetal dose is >10 rad; however, when radiation is directed to the breast, the fetal dose is lower than the teratogenic range by several orders of magnitude.[29] In our study we found no malformations either in women who delayed treatment or in those who did not delay treatment. However, our cohort was not delivered of babies who were exposed to chemotherapy during embryogenesis, when major malformations would occur. Babies exposed to cancer chemotherapy later in pregnancy do not appear to have a higher risk for dysmorphologic features.[26, 30]

Children born to women with breast cancer were significantly more likely to be preterm, mainly because elective cesarean sections were done more often to allow earlier start of cancer therapy. Of importance, our data are the first to document small birth weight for gestational age associated with breast cancer, which may be explained by the disease itself and by its treatment. The higher rate of stillbirth in pregnancy further suggests suboptimal intrauterine conditions; the association between small birth weight and stillbirth has long been recognized.[31]

Because the risk of metastatic disease is higher during pregnancy, it is important that pregnant women are instructed to perform regular breast self-examinations or that physicians caring for them perform the examinations during prenatal visits, with particular attention to any changes not consistent with pregnancy. Although breast cancer screening and treatment have advanced in the 30 years covered by this study, we cannot dismiss the importance of our results.

Because of the higher risk for stillbirth and small birthweight, women with breast cancer should undergo close follow-up by a high-risk obstetric unit to define the optimal time of delivery.

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Early menopause in long-term survivors of cancer during adolescence

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OBJECTIVE: We attempted to investigate the risk of early menopause after treatment for cancer during childhood or adolescence.

STUDY DESIGN: We interviewed 1067 women in whom cancer was diagnosed before age 20, who were at least 5-year survivors, and who were still menstruating at age 21. Self-reported menopause status in survivors was compared with that in 1599 control women.

RESULTS: Cancer survivors, with disease diagnosed between ages 13 and 19, had a risk of menopause four times greater than that of controls during the ages 21 to 25; the risk relative to controls declined thereafter. Significantly increased relative risks of menopause during the early 20s occurred after treatment with either radiotherapy alone (relative risk 3.7) or alkylating agents alone (relative risk 9.2). During ages 21 to 25 the risk of menopause increased 27-fold for women treated with both radiation below the diaphragm and alkylating agent chemotherapy. By age 31, 42% of these women had reached menopause compared with 5% for controls.

CONCLUSION: Treatment for cancer during adolescence carries a substantial risk for early menopause among women still menstruating at age 21. Increasing use of radiation and chemotherapy, together with the continued trend toward delayed childbearing, suggests that these women should be made aware of their smaller window of fertility so that they can plan their families accordingly. (AM J OBSTET GYNECOL 1992;166:788-93.)

Key words: Menopause, childhood cancer, radiation, alkylating agent, ovarian failure, follow-up studies

Once bleak, the outlook after diagnosis of cancer during childhood or adolescence has improved greatly in the last 20 years. Five-year relative survival rates in the United States are nearly 90% after Hodgkin's disease and 73% for acute lymphocytic leukemia diagnosed during childhood.[1] However, the toxic side effects of successful therapy mean that childhood cancer survivors face the prospect that the cancer could still adversely affect their lives, including their fertility.

In adults cancer treatment significantly reduces reproductive potential. Radiation to the gonads and chemotherapy, especially with alkylating agents, have been linked to gonadal dysfunction, primary and secondary amenorrhea, and azoospermia.[2-6] The probability of amenorrhea increases with age at treatment; older women are more likely than younger women to cease menstruating permanently.[2, 4] Reports of primary amenorrhea,[7, 8] severely damaged ovaries on autopsy,[9] altered timing of menarche,[10-12] and abnormal endocrine values in early adolescence after leukemia[11, 12] show the susceptibility of the prepubertal ovary to some level of damage from cancer therapeutic agents. On the other hand, an analysis of the effects of cyclophosphamide highlighted the resistance of the prepubertal ovary to damage relative to older girls and boys.[13]

No study has yet examined the risks for early menopause after cancer treatment during childhood or adolescence where the effects of different agents could be separated. As part of an interview study of survivors of all types of childhood or adolescent cancer, we examined several questions relating to the risk for early menopause. Specifically we sought to measure the pres-

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ence and extent of excess risk, to see whether the risk declined with time since treatment, and to examine the influence of different types of treatment on early menopause.

Patients and methods

Identification of subjects. Five cancer registries identified survivors and controls as described elsewhere.[14] Each registry identified all former cancer patients who met the following requirements for eligibility: a histologically diagnosed malignant neoplasm (or clinically diagnosed central nervous system neoplasm); first diagnosis from 1945 through 1974; 5-year survival after diagnosis at <20 years of age, and survival to ≥21 years of age or more by December 31, 1979. For this report we assembled a group of female controls who had survived to ≥19 years of age as of December 31, 1979, and who were at least age 21 years at interview by selecting up to two full siblings or half siblings closest in age to the survivors in our study.

We defined the age at cohort entry as the age at which a woman survived 5 years beyond diagnosis or reached age 21, whichever was later. For instance, a woman diagnosed at age 19 became eligible for cohort entry at age 24. Survivor cohort is defined as those members of the entire survivor population who were menstruating at the age of cohort entry (n = 954; Table I). The control cohort consisted of all control women who were still menstruating at age 21 (n = 1596; Table I). Nineteen women (18 survivors and one control) never menstruated and are not included in the analysis.

Data collection. Interviews were conducted by trained interviewers between 1980 and 1983 and lasted 45 to 60 minutes. Next-of-kin proxies were interviewed for 12% of survivors and 4% of controls. The most frequent reason for use of a proxy was death of the subject. Ninety-one percent of both groups agreed to be interviewed. At follow-up, 10% of the survivors and 1% of the controls had died. Survivors were aged 32.3 years on average at follow-up, and diagnosis had occurred on average at age 13.6. Controls averaged 33.0 years old at follow-up.

The questionnaire covered demographic and social characteristics and medical, reproductive, and menstrual histories. Each woman was asked her age at the time of her first menstrual period and whether her menses had started spontaneously. Menstrual status and age at menopause were determined from responses to the questions, "Are you still having menstrual periods?" and "What age did you have your last menstrual period?" At the end of the survivor's interview, permission was sought to locate medical records, including investigations of infertility, and to contact the siblings closest in age. A nearly identical interview was conducted with controls. Information on the survivor's tumor and treatment was abstracted from the tumor registry's record and other hospital records. The information abstracted included site and dose of radiotherapy, names of individual chemotherapeutic agents, but not total dose or number of cycles.

Table I. Women grouped according to menopausal status at follow-up

	Survivors	*Controls*
Women who became menopausal before they were eligible for the cohort	94	2
Women who became menopausal after cohort entry but before the end of follow-up	123	222
Women who were still menstruating at the end of follow-up	831	1374

Entry into the cohort occurred at age 21 or at 5-year survival, whichever came later.

Each woman's report of whether she was still having menstrual periods became our definition of menopause. In this paper we do not consider the issues of secondary amenorrhea or temporary cessation of menses, nor do we deal with menstrual problems or whether menopause was natural or the result of surgery.

Survivors were classified by treatment received; many had had only surgical treatment. For some, surgery meant immediate menopause; for instance, a total abdominal hysterectomy for a malignant ovarian tumor. Radiation was categorized according to whether it was administered above or below the diaphragm; chemotherapy was divided into alkylating or nonalkylating types.

Statistical methods. Standard contingency table methods, including χ^2 tests, were used to analyze associations between characteristics of interest and menopause.

Risks were calculated as the number of events (women reaching menopause) divided by the estimated person-years of follow-up. Relative risks (RR) refer to the ratio of risk in a treated group to that in controls. Associated tests of significance were based on the assumption that hazard rates were constant within 5-year age intervals.[15] This is an open cohort because some women were not at risk at age 21, but became so at later ages when they had first survived treatment for 5 years. Methods of survival analysis were used to estimate the proportion still menstruating among the survivor cohort. Appropriate modifications of the usual actuarial methods of analysis were used.[16, 17] Calculations were carried out with *Epicure*,[18] a package of programs developed for data analysis on the personal computer, and SAS (Statistical Analysis Systems, Inc., Cary, N.C.) for mainframe data analysis. The proportional

Table II. Survivors who were postmenopausal at cohort entry grouped according to factors associated with cancer

Cancer factors	*Not menstruating/total*	%	*p**
All	94/1048	9	
Cancer site or type			0.001
Female genital cancer	52/90	58	
Hodgkin's disease	20/206	10	
Non-Hodgkin's lymphoma	3/31	10	
Soft-tissue sarcoma	8/115	7	
Leukemia	1/15	7	
Brain or CNS tumors	5/133	4	
Bone cancer	1/65	2	
Other cancers	4/393	1	
Treatment			0.001
Surgery only	24/493	5	
Sterilizing surgery + RT-CT	25/25	100	
RT only	13/261	5	
CT only	4/68	6	
RT above diaphragm + AA	3/38	8	
RT below diaphragm + AA	23/79	29	
Other treatments	2/84	2	
Age at diagnosis (yr)			0.001
0-12	4/286	1	
13-14	7/134	5	
15-19	83/628	13	

CNS, Central nervous system; *RT*, radiotherapy; *CT*, chemotherapy; *AA*, alkylating agent chemotherapy.

*χ^2 test for homogeneity.

hazards model of Cox[19] was not used because hazard ratios changed with age.

Results

Menopause before age at cohort entry. Ninety-four (9.0%) of the 1048 survivors who had commenced menstruating had reached menopause by age at cohort entry; many became menopausal immediately on treatment (Table II). Over half of the 90 women treated for genital cancer were no longer menstruating; treatment for 25 women included removal of the genital organs. All women treated for thyroid cancer, kidney cancer, melanoma, neuroblastoma, or retinoblastoma were still menstruating at entry into the cohort. For other forms of cancer the proportion no longer menstruating ranged from 2% for bone cancer survivors to 10% for survivors of either Hodgkin's disease or non-Hodgkin's lymphoma. Women who were treated with infradiaphragmatic radiation plus alkylating agent chemotherapy were nearly four times more likely to be menopausal than women who had radiotherapy *above* the diaphragm with alkylating agents. The risk of menopause increased significantly with increasing age at diagnosis.

Two of the 1593 controls were menopausal at age 21. One had Down syndrome; she had entered menarche normally, but had very heavy menstrual bleeding that was treated by hysterectomy. The other woman had very irregular periods for 9 years and was taking birth control pills; there was no specific reason for menopause.

Table III. Age-specific relative risks of menopause for women menstruating at cohort entry grouped according to age at diagnosis and type of cancer

	Age intervals		
	21-30 (yr)	*31-40 (yr)*	*≥41 (yr)*
Controls			
Rate*	5.40	22.53	63.92
n	68	99	55
Survivors			
Age at diagnosis			
0-12			
Relative risk	0.62	0.53	—
n	7	6	0
13-19			
Relative risk	2.32‡	0.78	0.98
n	58	33	19
Cancer site and treatment			
Hodgkin's disease			
Relative risk	3.35‡	1.27	2.93
n	21	8	3
RTb + AA			
Relative risk	9.57‡	—	—
n	10	0	0
Other treatments			
Relative risk	2.11†	1.34	2.93
n	11	8	3
All other cancers			
Relative risk	1.46	0.65	0.81
n	44	31	16
RTb + AA			
Relative risk	8.56‡	4.23	—
n	4	1	0
Other treatments			
Relative risk	1.35	0.64†	0.81
n	40	30	16

RTb + AA, Radiotherapy below the diaphragm plus alkylating agent chemotherapy.

*Menopause rates per 1000 person-years; *n* denotes the number of menopause events; relative risks compare the risk that menopause will occur within that age interval for survivors versus controls and are obtained by dividing the rate for survivors by the rate for controls.

†Significance level of $0.01 < p < 0.05$.

‡$p \leq 0.01$.

Menopause in the survivor and control cohorts. Among women who were still menstruating when they entered the cohort, several interesting relative risk patterns emerged within specific age intervals after cohort entry (Table III). Women diagnosed after age 12 years were significantly more likely to become menopausal than controls during the age interval 21 through 30 years (RR 2.32; CI 1.63 to 3.29). Women diagnosed between ages 0 and 12 were not at greater risk than controls and do not appear in Tables IV and V. Women with Hodgkin's disease had significantly elevated risks of menopause mainly during the first 10 years after

cohort entry (RR 3.35; CI 2.06 to 5.47), especially if treated with radiation below the diaphragm plus alkylating agents (RR 9.57; CI 4.93 to 18.69). The relationship between Hodgkin's disease and treatment is discussed below.

Table IV presents the main findings from the study. The age-specific menopause rate was four times greater (RR 4.32; CI 2.28 to 8.17) for survivors overall than for controls from ages 21 through 25. At later ages the relative risk did not differ significantly overall between survivors and controls. Thus the main excess risk among survivors occurred early.

The menopausal rate for women treated with surgery alone was not significantly different from the rate for controls either overall or at any age interval. For other treatments the risk of menopause relative to controls was high at younger ages and declined as the women aged. Women treated with alkylating agent chemotherapy alone had nine times (RR 9.17; CI 2.67 to 31.49) the rate of menopause of controls. Treatment with radiotherapy alone increased the rate over controls by almost fourfold (RR 3.66; CI 1.34 to 9.99). The greatest increase in risk occurred in women who were treated with radiation below the diaphragm plus alkylating agents (RTb + AA); during their early 20s these women had about 27 times the rate of menopause as controls (RR 27.39; CI 12.42 to 60.35). For some types of therapy, for instance, for radiotherapy alone and RTb+AA, the excess risk persisted into the later 20s though at a lower level. Our data are too sparse to say much about persistence of risk into the 30s.

To see if treatment accounted for the increased risk after Hodgkin's disease (Table III), these women and those with other diagnoses were stratified as to whether the treatment was RTb + AA. The risk of menopause remained high in survivors treated with RTb + AA and much lower in survivors with other treatments, regardless of the type of cancer. Thus the elevated risk for women treated for Hodgkin's disease resulted from their exposure to RTb + AA.

The median age at menopause for women treated with surgery alone between the ages of 13 and 19 was 47 compared with 49 years for controls (Fig. 1). Women treated with RTb + AA reached menopause at a median age of about 32 years (obtained by extrapolation), compared with about 44 years for women treated with radiotherapy alone or other therapies.

The conditional probabilities highlight the strong effect of RTb + AA treatment of adolescent girls on menopause during ages 21 through 30 years (Table V). Women who entered the cohort still menstruating faced about a 40% chance of reaching menopause before age 31; their risk beyond age 30 was unknown, because the last menopause in this treatment group occurred at age 30. The conditional probability of menopause between ages 31 and 40 among women who were menstruating at age 30 was not different among controls and survivors in various treatment groups.

Table IV. Age-specific menopause rates and relative risks* for women who were menstruating at cohort entry grouped according to treatment

	Age interval (yr)			
	21-25	*26-30*	*31-40*	*41+*
Controls (n = 22)				
Rate	2.18	9.91	22.53	63.92
All survivors (n = 110)				
Rate	9.41	15.98	17.49	62.91
Relative risk	4.32†	1.61	0.78	0.98
Relative risk according to survivors' treatment				
Surgery alone (n = 48)	1.12	1.11	0.71	0.84
Radiation alone (n = 33)	3.66†	2.41†	0.90	1.22
RTb + AA (n = 14)	27.39‡	4.64‡	—	—
Alkylating agents alone (n = 5)	9.17‡	—	—	—
All other treatments§ (n = 10)	3.30	1.99	1.17	—

All women were diagnosed between ages of 13 and 19 years.

*Menopause rates per 1000 person-years. Relative risks compare the menopause rate in each age interval for survivors versus controls; they were obtained by dividing the rate for survivors by the rate for controls and were not calculated if the number of women reaching menopause *(n)* was <3 for any one category.

†Significance level of $0.01 < p < 0.05$.

‡$p \leq 0.01$.

§Includes treatments with nonalkylating agent chemotherapy alone and alkylating agent chemotherapy alone.

Comment

We found that women who survived cancer for which they were treated between the ages of 13 through 19 reached menopause earlier than controls, except for cancer survivors treated only with surgery. This finding applies to women who were menstruating at 21 or as soon as 5 years had passed since diagnosis, whichever was later. An elevated risk of menopause was most pronounced in women who were treated with modern combined alkylating agent chemotherapy and radiation below the diaphragm; however, the higher risk of menopause was also seen in those treated with radiotherapy alone and with alkylating agent chemotherapy alone. The excess risk was highest during the years 21 to 25 and diminished as women aged.

This is the first report to describe the risks associated with different therapies on menstrual functioning in long-term survivors of adolescent cancer. Earlier case reports indicated that pelvic radiotherapy in prepubertal girls can cause both primary and secondary ovarian failure[7]; studies have linked ovarian damage to chemotherapy for childhood brain tumors and possibly

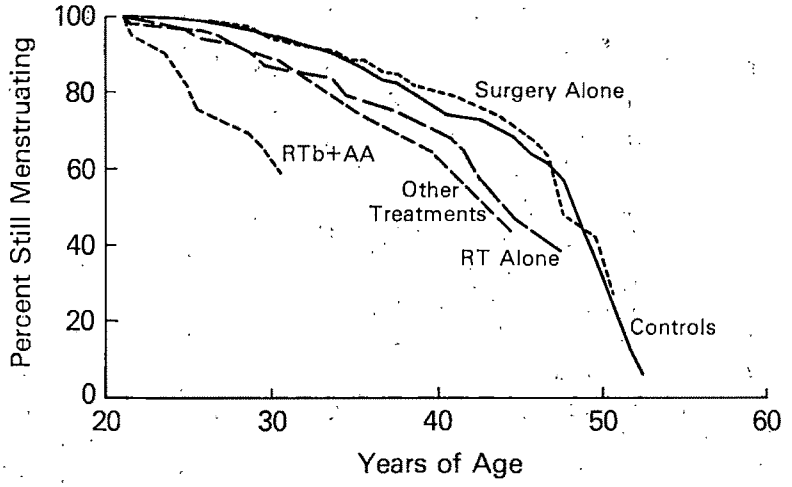


Fig. 1. Proportion still menstruating among cancer survivors diagnosed between ages 13 and 19 grouped by type of treatment received compared with proportion of controls still menstruating (Kaplan-Meier curves). Survivor and control cohorts only. *RTb+AA*, Radiotherapy below diaphragm plus alkylating agents.

Table V. Conditional probability of menopause* (%) among members of the survivor and control cohorts (cancer diagnosis between ages 13 and 19)

	Ages 21-30 (%)	*Ages 31-40*
Controls	5.7 (4.8, 6.7)	21.5 (18.6, 24.3)
Survivors by type of treatment		
Surgery alone	6.3 (4.2, 8.4)	15.1 (10.4, 19.5)
RTb + AA	41.5 (26.6, 53.3)	Not calculated
Other	13.1 (9.9, 16.6)	21.6 (13.0, 29.5)

*Probability that a woman who is menstruating at the beginning of each age interval will reach menopause within the interval. *Not calculated* indicates there were no events in 44 person-years. *Numbers in parentheses* are 95% confidence intervals.

after cisplatin administration.[20, 21] Studies of adult women treated for Hodgkin's disease[3-5] showed dose- and age-dependent ovarian failure after treatment with chemotherapy and radiation. In a previous article on the effects of cancer therapy on fertility (defined as the first recognized pregnancy after marriage) in this population, we reported that female fertility was unaffected by treatment with alkylating agents.[14] This finding is consistent with early menopause in women where ovarian failure occurred some time after treatment.

These risks may not apply to adolescents treated today because of better focusing of radiation beams and because shielding of the ovaries is more common. On the other hand, the current widespread use of multiagent regimens for most cancers, including those of childhood, suggests the presence of considerable future risk for early cessation of menses for those treated at susceptible ages.

Diagnosis and treatment before puberty is less damaging to the ovary than treatment after puberty.[13] In our study the small numbers of events in women diagnosed at <13 years of age (n = 13; Table III) made detailed analysis impossible. Among the possible reasons for the apparently reduced risk seen here is bias because of selection, first for the study and then for this analysis. Women diagnosed at <13 years of age had to have reached age 21 years with menstrual function still intact. Treatment before puberty obviously has an adverse impact on ovarian function in this group, because 18 women who were treated before puberty never started menstruating.

Among women who reached menopause at <21 years of age who were not treated with sterilizing surgery (Table II), the increased risk was associated with older age at diagnosis and combined radiotherapy with alkylating agents just as in the survivor cohort.

Although the control group was not chosen to be representative of the general population, the proportion reaching menopause was similar to that reported previously in a cohort of healthy women.[22]

Amenorrhea may occur for many reasons, including hypergonadotropic hypogonadism, resistant ovary syndrome, and poor nutritional status. In this large, multicenter study we did not attempt to confirm menopause by gonadotropin levels. We relied on self-reports, which in other studies have proved to be quite valid, especially where menopause had occurred in the recent past.[23]

If fertility is unimpaired, survivors of childhood or adolescent cancer should not be overly anxious about the success of a pregnancy or the health of a child born after treatment has concluded.[24, 25] However, the same treatments that lead to reduced fertility are also implicated here with early menopause.

This report describes the risks for women who were long-term survivors of childhood or adolescent cancer and who had survived to reach ≥21 years of age. Besides postmenopausal changes leading to osteoporosis and other problems, early menopause obviously influences childbearing. Delayed childbearing became more common in the United States in the late 1980s, and the average age at which women have their first child continues to rise.[26] As multiagent therapies prove to be more effective against cancer, their use is expanding. Our data suggest that early menopause will occur more frequently as girls treated today come into their reproductive years. A woman with a history of adolescent cancer for which she received both radiation and chemotherapy needs to understand her cancer experience and its consequences. Her window of fertility may be much smaller than her sisters'.

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Erythromycin therapy in preterm premature rupture of the membranes: A prospective, randomized trial of 220 patients

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OBJECTIVES: The use of prophylactic antibiotics in the management of preterm premature rupture of the membranes has not been adequately studied. The purpose of this study was to evaluate the efficacy of oral erythromycin therapy in the prolongation of latency and reduction of infectious morbidity after preterm premature rupture of membranes.

STUDY DESIGN: In this randomized, prospective, double-blind, placebo-controlled study, 220 women at 20 to 35 weeks' gestation were evaluated. Subjects received oral erythromycin 333 mg (n = 106) or indistinguishable placebo (n = 114) every 8 hours from randomization to delivery.

RESULTS: Prolongation of latency was identified with erythromycin therapy (p = 0.02), particularly for those destined to have chorioamnionitis (p = 0.003) and those with oligohydramnios (p = 0.01). No decrease in the incidence of maternal or neonatal infectious morbidity was seen.

CONCLUSIONS: Oral erythromycin delays, but does not prevent, the onset of clinical infection when administered to women with preterm premature rupture of membranes. This regimen does not decrease neonatal morbidity and mortality. (Am J Obstet Gynecol 1992;166:794-802.)

Key words: Preterm premature rupture of fetal membranes, erythromycin, antibiotics, infection

Preterm premature rupture of the membranes is a major cause of maternal, fetal, and neonatal morbidity.[1-3] Neonatal morbidity is mainly related to preterm birth as a result of the short latency from membrane rupture to delivery. Demographic studies have also demonstrated a correlation between preterm premature rupture of the membranes and maternal and neonatal infection, and neonatal sequelae have been demonstrated to increase with maternal infection before delivery.[2-5]

There have been several studies (controlled and uncontrolled) describing the use of antibiotics in the management of patients with preterm premature rupture of the membranes. In these studies demethylchlortetracycline,[6] vaginal nitrofurazone,[7] penicillin, kanamycin,[8] and ampicillin[9, 10] have been evaluated for their efficacy in reducing maternal and neonatal morbidity. Unfortunately, these studies have included heterogeneous groups of patients at various gestational ages with various risk factors. In addition, some of the agents studied are not currently considered appropriate for use during pregnancy.[6, 8] Moreover, these studies have been nonblinded[9, 10] or retrospective[11] or have demonstrated no clinical efficacy.[7]

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In a recent prospective, double-blind study the use of parenteral mezlocillin followed by oral ampicillin until delivery was associated with prolonged latency, increased birth weight, and reduced incidences of chorioamnionitis, endometritis, and suspected neonatal sepsis.[12] In addition, a prospective study conducted at this institution by Amon et al.[9] found that the combination of parenteral and oral ampicillin in patients with premature rupture of the membranes was associated with an increased latency period and reduced neonatal infectious morbidity. At the time of submission of this article there are no published randomized studies of the use of erythromycin in patients with preterm premature rupture of the membranes.

The purpose of this prospective, double-blind, placebo-controlled study was to assess whether the use of oral antibiotic therapy in women with preterm premature rupture of the membranes would increase the latency period and reduce the incidence of maternal and neonatal infectious morbidity.

Material and methods

This clinical trial was performed at the University of Tennessee, Memphis, a tertiary care perinatal referral center, from March 1, 1989, to Aug. 1, 1990. The study was approved by the university's institutional review board.

Each woman who was admitted with a history of preterm premature rupture of the membranes had a sterile speculum examination for confirmation of membrane rupture and evaluation of cervical dilatation. Mem-

brane rupture was confirmed by the visualization of amniotic fluid passing from the cervical os or by the presence of a pool of fluid in the posterior fornix that gave positive results with Nitrazine or ferning. Cervical cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* and vaginal cultures for group B streptococci were obtained at the time of initial examination. Digital vaginal examinations were not performed in the absence of advanced cervical dilatation. Gestational age was determined by menstrual history, early clinical examination, or ultrasonographic assessment before 24 weeks' gestation. Fetal heart rate and uterine contraction monitoring was performed for evidence of fetal distress and labor. Blood was drawn for complete blood cell count and C-reactive protein. Ultrasonographic examination was performed for estimation of fetal weight, amniotic fluid assessment, and amniocentesis. Amniotic fluid from amniocentesis was assessed for pulmonary maturity (fetal surfactant index ≥47, lecithin/sphingomyelin ratio ≥2:1 considered mature), Gram stain, culture, and sensitivity. Vaginal pool fluid was sent for pulmonary maturity studies (fetal surfactant index) if amniocentesis was not feasible or was unsuccessful. Catheterized urine was obtained for microscopy, culture, and sensitivity. Women were excluded from study in the presence of the following: membrane rupture of >72 hours' duration, cervical dilatation >4 cm, progressive labor, vaginal bleeding, temperature ≥99.0° F, active infection requiring antibiotic therapy, antibiotic therapy within 1 week before admission, active hepatic disease, erythromycin allergy, cervical cerclage, or a medical condition necessitating delivery. Women with fetuses having intrauterine growth retardation (<10th percentile), congenital anomalies, or evidence of fetal distress were also excluded from randomization. Women requiring parenteral tocolysis for preterm labor on admission were excluded from randomization unless tocolysis was successful.

Consenting women between 20 weeks' and 34 weeks' 6 days' gestation were then randomized. The randomization scheme was generated by computerized random number tables and administered by the inpatient pharmacy at this institution. To reduce the chance of differing gestational ages within the two study groups, randomization was stratified at 30 weeks' gestational age. The enteric-coated erythromycin base and placebo were supplied by Boots Pharmaceuticals (Lincolnshire, Ill.) and packaged by the inpatient pharmacy in indistinguishable plastic wraps. At the time of randomization the patients were assigned by the pharmacist to receive either erythromycin base, 333 mg, or an indistinguishable placebo tablet every 8 hours until delivery. All investigators, patient care givers, and patients were blinded to the randomization scheme until after completion of the study and data analysis.

Subsequent to a minimum of 12 hours of observation, patients not showing evidence of infection, fetal distress, or infection were transferred to the antepartum ward. They were restricted to bed rest with bathroom privileges and were counseled to remain in the hospital until delivery. Digital pelvic examinations were prohibited until such time as a decision was made to allow delivery. Women with immature pulmonary maturity studies (fetal surfactant index <47 or lecithin/sphingomyelin ratio <2:1) received betamethasone, 12 mg intramuscularly for two doses, 24 hours apart. Patients had follow-up with nonstress tests and biophysical profile testing daily for 5 days with subsequent twice-weekly testing if results remained reassuring (defined as a reactive nonstress test or a biophysical score of ≥6/8). The nonstress test was considered reactive if there were two accelerations of 15 beats/min lasting 15 seconds over a 20-minute period. The biophysical profile score was obtained over a 30-minute period with a score of 0 or 2 for each of fetal breathing activity, movement, tone, and amniotic fluid pocket of 2 × 2 cm. Complete blood cell count and C-reactive protein were obtained daily for 5 days and then twice weekly.

In the presence of labor or suspected chorioamnionitis, patients were transferred to the labor unit for continuous maternal and fetal evaluation. Chorioamnionitis was defined as a maternal temperature of ≥100.4° F (in the absence of another identifiable source of infection) plus two of the following additional diagnostic criteria: fetal or maternal tachycardia, uterine contractions or tenderness, or foul-smelling amniotic fluid. Suspected chorioamnionitis was diagnosed when only two of the three diagnostic criteria for chorioamnionitis were met. Parenteral tocolysis was used therapeutically for preterm labor in the absence of clinical evidence of infection. Patients with progressive labor refractory to parenteral tocolysis, abnormal fetal heart rate patterns, nonreassuring fetal testing (nonreactive nonstress test with biophysical profile score ≤4/8), and suspected or frank chorioamnionitis were allowed to deliver. Cesarean delivery was performed for standard obstetric indications. Intravenous ampicillin and gentamicin were administered for intrapartum temperature >100.4° F and continued until the patient remained afebrile for 48 hours. Endometritis was diagnosed in the presence of a persistent temperature of ≥100.4° F with abnormal uterine tenderness, in the absence of another identifiable source of infection.

Neonates were cared for by neonatal subspecialists blinded to the maternal treatment arm. Preterm infants who were delivered >48 hours after membrane rupture and any infant of a mother with intrapartum fever received parenteral ampicillin and gentamicin, pending results of blood cultures and urine Wellcogen testing for group B streptococci; daily complete blood cell count and C-reactive protein testing were also done. Antibiotics were then discontinued if the infant re-

Table I. Maternal demographic findings on admission

	Placebo (n = 114)	*Erythromycin (n = 106)*
Race (% black)	74.6	75.5
Age (yr, mean ± SD)	24.1 ± 5.6	23.7 ± 5.7
Weight (lb, mean ± SD)	159 ± 36	156 ± 37
Gravidity (median ± SD)	2 ± 1.4	3 ± 1.8
Gestation at premature rupture of membranes† (wk, mean ± SD)	29.8 ± 3.6	30.3 ± 3.4
Latency from premature rupture of membranes to admission (hr, mean ± SD)	13.9 ± 17.7	13.2 ± 18.7

Table II. Maternal morbidity after conservative therapy of preterm premature rupture of membranes at 20 to 34 weeks' gestation

	Placebo (n = 112) (%)*	*Erythromycin (n = 105)* (%)*
Chorioamnionitis	19.6	17.1
Suspected chorioamnionitis	14.3	17.1
Endometritis	6.3	6.7
Cesarean delivery	16.1	24.8
Abruptio placentae	9.8	14.2

*Three patients lost to follow-up (placebo, $n = 2$; erythromycin, $n = 1$).

mained clinically stable, without hypothermia, elevated C-reactive protein level, or increased or decreased white blood cell count. Neonatal sepsis was diagnosed when the infant appeared clinically infected, and either cultures (blood, urine, or cerebrospinal fluid) were positive or radiographic findings were diagnostic (pneumonia, necrotizing enterocolitis). Suspected neonatal sepsis was diagnosed when clinical findings of sepsis could not be confirmed on microbiologic or radiographic assessment or in the presence of an abnormal white blood cell count or C-reactive protein level in an otherwise healthy-appearing neonate. Infants diagnosed as having suspected or confirmed sepsis received a full course of parenteral antibiotics. Cranial ultrasonographic examinations were routinely performed on all infants weighing <1750 gm and on any infant demonstrating suspicious neurologic findings.

Statistical analysis was performed by a statistician blinded to the study code with the SAS system for elementary statistical analysis (SAS Institute Inc., Cary, N.C.). All patients were maintained within their randomization group for analysis, regardless of compliance and results of cultures performed on admission. Comparative data analysis was performed with Fisher's exact test and the Student t test where appropriate. Life-table analysis with the Wilcoxon statistic was performed for temporal data. A p value of <0.05 was considered statistically significant.

Results

A total of 268 patients were eligible for the study. Eighteen eligible women refused to participate (7.6%), and 30 were not screened (11.2%). The other 220 women were randomized to receive either a placebo ($n = 114$) or oral erythromycin ($n = 106$). Six women did not complete the protocol (four receiving erythromycin), and three of these were lost to follow-up after they left the hospital against medical advice (one receiving erythromycin). Available data from the three women not completing the protocol were included in the statistical analysis. An additional 10 women received ampicillin (seven receiving erythromycin) for urinary tract infection. There were six sets of twins (four receiving erythromycin). Parenteral tocolysis was administered in equal numbers of women in both placebo and erythromycin study groups (59%). Parenteral tocolysis (subcutaneous terbutaline 61.7%, intravenous magnesium sulfate 49.2%, ritodrine 10.9%). There was no difference between the two study groups in the distribution of tocolytic agents used. Two or more tocolytic agents were used in 33.3% of the placebo tocolysis group and in 22.6% of the erythromycin tocolysis group (not significant). Betamethasone was administered to 50.9% of women in the placebo group and 57.5% of women in the erythromycin group.

Table I compares the demographic distribution and clinical findings between the erythromycin and placebo groups. There were no significant differences in any of the parameters studied. Fifteen patients had gastrointestinal discomfort (8/15 receiving placebo), but none required discontinuation of the study medication.

Cervical and vaginal cultures were performed in 188 of 220 women on admission (85.5%). No significant differences were identified with respect to carriage of *N. gonorrhoeae, C. trachomatis,* or group B streptococci in the placebo and erythromycin groups (8.2% vs 6.6%, 16.3% vs 23.0%, and 15.4% vs 18.4%, respectively). Amniocentesis was attempted in 113 patients (53.6%) and was successful in 97 (44.1%). Of the remaining patients, two refused the test and 100 had severe oligohydramnios precluding the procedure. The incidence of positive amniotic fluid cultures in the placebo group (17.9%) and erythromycin group (18.4%) was similar.

Table II summarizes maternal morbidity related to premature rupture of the membranes. The incidence of clinical abruptio placentae was surprisingly high in both groups. No differences in the incidences in clinical chorioamnionitis or endometritis were identified.

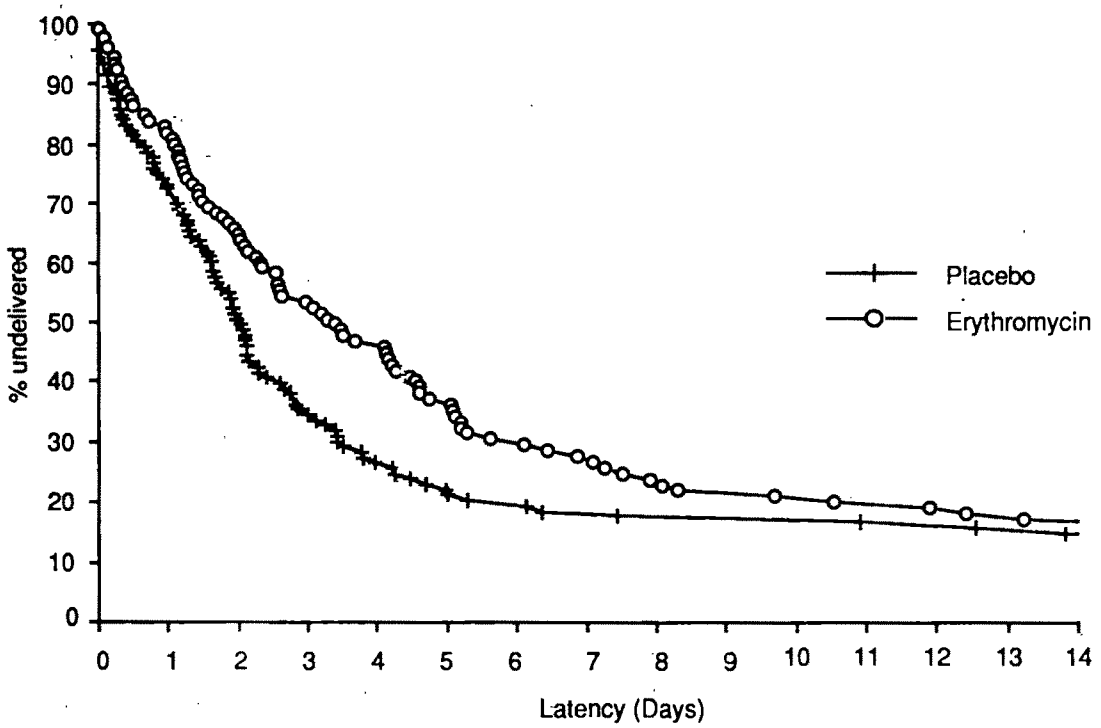


Fig. 1. Latency from randomizat on to delivery for patients receiving erythromycin base or placebo.

Table III. Women remaining undelivered on each of the first 7 days after randomization

	Women (No.)						
	1 Day	*2 Days*	*3 Days*	*4 Days*	*5 Days*	*6 Days*	*7 Days*
Erythromycin (n = 106)	87 (83%)	69 (65%)	58 (54%)	49 (46%)*	38 (36%)	30 (29%)	29 (27%)
Placebo (n = 114)	82 (72%)	57 (50%)	40 (35%)	30 (26%)	24 (21%)	21 (19%)*	20 (18%)
p Value	0.05	0.02	0.003	0.001	0.01	0.057	0.056

*One patient lost to follow-up.

Fig. 1 displays the survival curve for latency from randomization to delivery. Significant prolongation of latency from randomization to delivery (p = 0.02) was identified with erythromycin therapy. Significant differences in latency were noted in the first week after randomization (Table III). Subsequent to this time, no differences in latency were identified.

Fig. 2 displays the survival curves on the basis of the development of subsequent chorioamnionitis. The latency curve was significantly prolonged for patients with chorioamnionitis (p = 0.003), but statistical significance was not reached for the suspected chorioamnionitis group (p = 0.10) or for those not having infection (p = 0.43).

Fig. 3 displays survival curves for women with positive amniotic fluid cultures, negative cultures, and for those who had no amniocentesis because of oligohydramnios. Significant differences in latency period with erythromycin were noted only in those women not undergoing amniocentesis (p = 0.01). The incidence of chorioamnionitis was 12.5% and 29% for women with negative and positive amniotic fluid cultures with median latencies of 4.16 and 1.84 days, respectively. Chorioamnionitis was seen in 24% of women with oligohydramnios precluding amniocentesis, with a median latency of 2.12 days.

The 220 pregnancies resulted in 226 births (six sets of twins). Outcome data were not available for three infants of women who were lost to follow-up. A male predominance was identified in both groups (erythromycin 55.0%, placebo 58.8%).

Table IV compares perinatal outcome between the two study groups. There were seven stillbirths; however, only two of these occurred in infants weighing >600 gm (both in the placebo group). In addition, there were no significant differences in perinatal morbidity

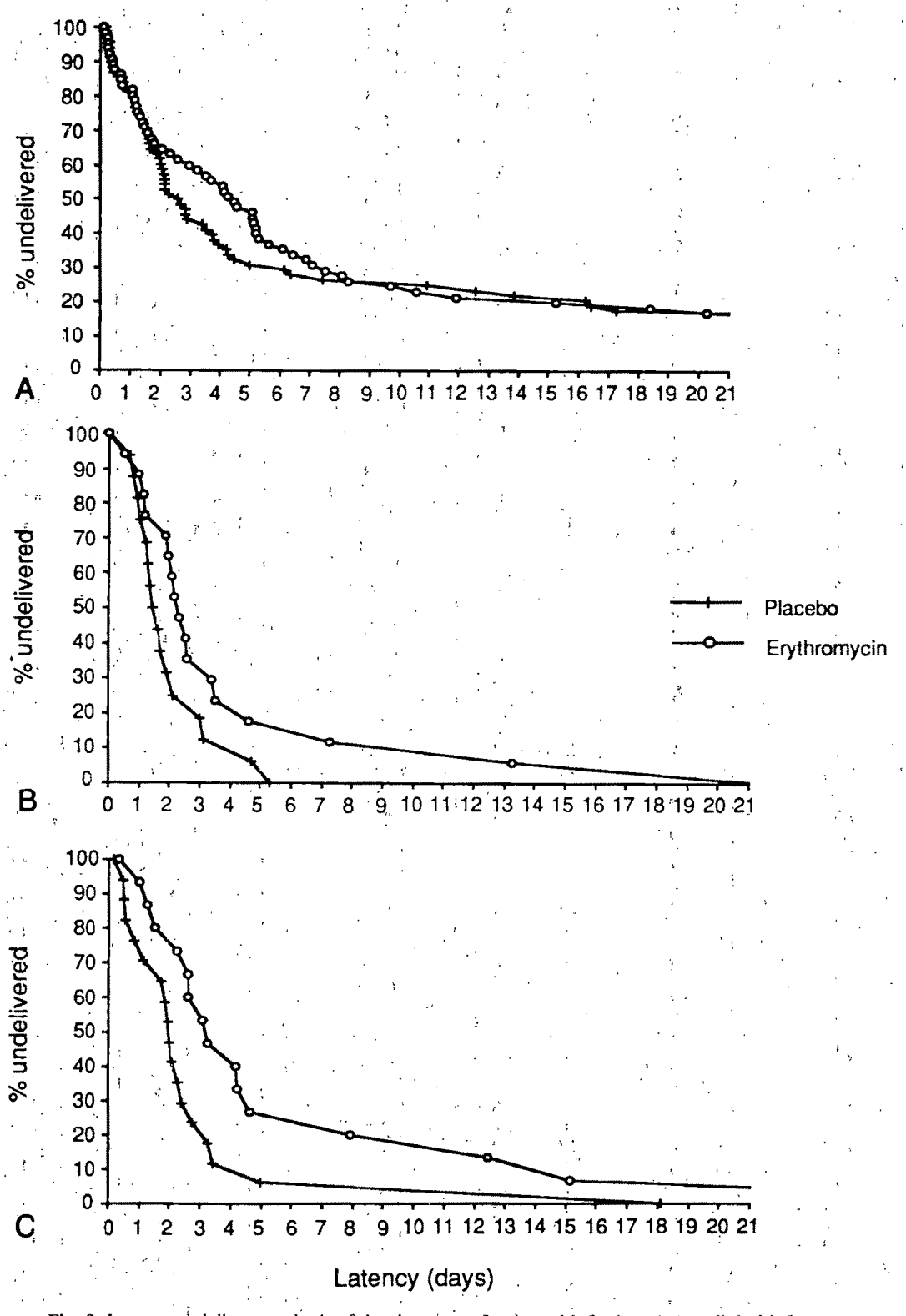


Fig. 2. Latency to delivery on basis of development of maternal infection. **A,** No clinical infection; **B,** suspected chorioamnionitis; **C,** clinical chorioamnionitis.

and mortality between the two groups. There were four cases of congenital pneumonia in the placebo group: Two had positive cultures for group B streptococci, *Enterobacter cloacae* grew in one infant, and one case was diagnosed radiographically after negative cultures.

Bacteria grown from amniotic fluid and early neonatal blood cultures are listed in Table V.

Table IV. Perinatal outcomes

	Placebo		*Erythromycin*	
	No.	*%*	*No.*	*%*
Stillbirth	5	4.4	2	1.8
Live birth	109	95.6	107	98.2
Fetal distress	12	11.0	13	12.1
5 min Apgar score <7	20	18.3	15	13.1
Birth weight (gm, mean ± SD)	1817 ± 637		1771 ± 653	
Sepsis	15	13.8	14	13.1
Suspected sepsis	22	14.7	22	20.2
Death from sepsis	3	2.8	1	0.9
Pneumonia	4	3.7	0	0*
Respiratory distress syndrome	24	22.0	27	25.2
Intraventricular hemorrhage	14	12.8	11	10.3
Necrotizing enterocolitis	12	11.0	8	7.5
Hospitalized >28 days	24	22.0	26	24.3
Survival	104	91.2	100	91.7

*$p = 0.06$.

Table V. Organisms recovered from amniotic fluid obtained by amniocentesis and early neonatal blood culture

Amniotic fluid	*No. of positive cultures*	*Neonatal blood*	*No. of positive cultures*
N. gonorrhoeae	3	Group B streptococci	4
Streptococcus viridans	3	*Streptococcus viridans*	3
Group B streptococci	2	*Enterococcus*	3
Acinetobacter wolffi	2	*Staphylococcus cureus*	2
Bacillus cereus	1	*Escherichia coli*	2
Escherichia coli	1	*Klebsiella*	1
Staphylococcus aureus	1	*Bacillus cereus*	1
Enterobacter cloacae	1	*Citrobacter*	1
Peptococcus	1	*Enterobacter cloacae*	1
Peptostreptococcus	1	*Staphylococcus epidermidis*	4
Bacteroides fragilis	1		
Fusobacterium	1		
Moraxella	1		
Staphylococcus epidermidis	3		

Comment

Several studies have attempted to address the role of antibiotic therapy in the prolongation of pregnancy and reduction of maternal and neonatal infectious outcomes. Unfortunately, these studies have been of small sample size,[9, 10, 12] nonblinded,[9, 10] or retrospective.[11]

In an excellent double-blind prospective evaluation of 85 women reported by Johnson et al.,[12] treatment with intravenous mezlocillin (followed by oral ampicillin until delivery) was associated with an increase in latency to delivery and a decrease in suspected sepsis. A 150% increase in the number of undelivered women was seen after 1 week of therapy. However, sample size was small. Although an increase in mean latency was claimed, median latency was not provided. It is generally accepted that in skewed population curves, such as latency after premature rupture of the membranes, the median is more reflective of population trends.

A prospective evaluation of 82 women with preterm premature rupture of the membranes from this institution has revealed a prolongation of latency and decrease in neonatal infectious morbidity with ampicillin therapy.[9] In this study the treated patients received intravenous ampicillin for 24 hours, followed by oral ampicillin until delivery, at which time parenteral therapy was reinitiated. Control patients received no antibiotics. An overall difference in survival curves was identified, but the nonblinded nature of this study may have introduced bias in patient management subsequent to randomization.

Morales et al.[10] reported on 165 women with preterm premature rupture of the membranes who were randomized into four different treatment schemes: no treatment ($n = 41$), steroids alone ($n = 43$), ampicillin alone ($n = 37$), or ampicillin and steroids ($n = 44$). There were no significant differences among the study groups with regard to mean latency period. The data were subsequently reanalyzed according to the use of antibiotics or steroids. There were no significant differences among these groups regarding gestational age at delivery or birth weight. Interestingly, the authors found differences of chorioamnionitis among the four

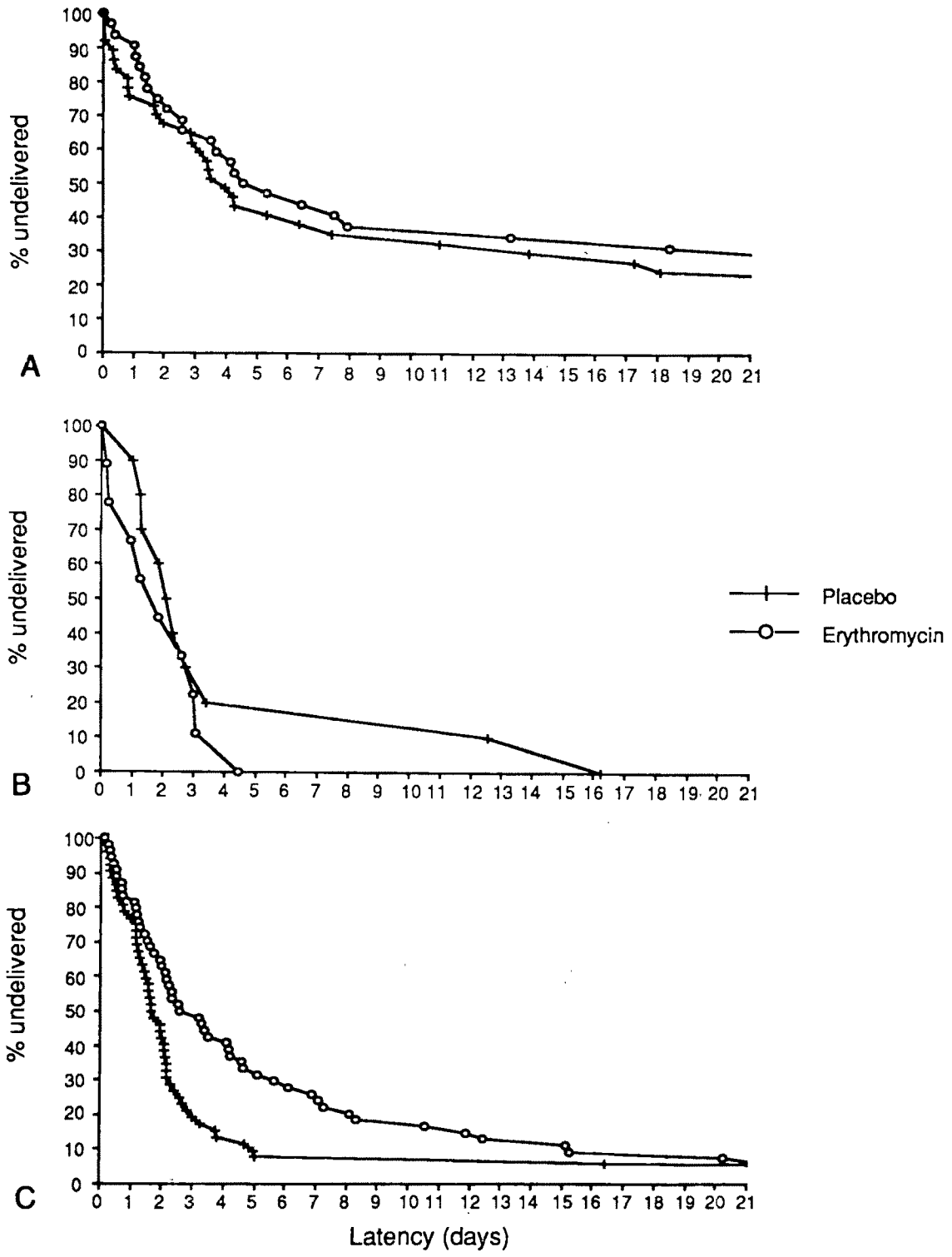


Fig. 3. Latency to delivery on basis of amniotic fluid culture results. **A,** Negative results of amniocentesis; **B,** positive results of amniocentesis; **C,** no amniocentesis (oligohydramnios).

study groups: lower incidences of neonatal sepsis in patients receiving ampicillin (with or without steroids) and a lower incidence of significant neonatal morbidities in the steroid-treated group (with or without ampicillin). This study was not blinded, and the sample size in each arm was small. Further, postrandomization exclusion was permitted. Finally, the results of this trial, randomized to multiple treatment arms, were analyzed

with univariate statistics. Thus it is difficult for us to come to any concrete conclusions on the basis of the presented data.

To our knowledge this is the first double-blind, randomized, placebo-controlled study with adequate sample size to assess the efficacy of oral erythromycin therapy in patients with preterm premature rupture of the membranes. We believed that a prolongation of latency by at least 1 week would be required to significantly reduce the incidence of neonatal sequelae. On the basis of the previous experience at this institution, we calculated that a sample size of 100 women would be required in each study arm to demonstrate a 20% increase in undelivered patients after 1 week of therapy (control group incidence of 29% undelivered, power = 0.80, $\alpha = 0.05$). As is outlined in Table II significant increases in the number of undelivered patients were obtained between 2 and 5 days after randomization. Although a trend toward pregnancy prolongation was seen after 7 days of therapy, this finding was not statistically significant ($p = 0.056$). Thus, although prolongation of latency is evident with erythromycin therapy, this effect is limited primarily to the first 5 days after initiation of treatment. Oral erythromycin therapy was not associated with a significant reduction in infant morbidity or mortality, although a trend toward a decrease in neonatal pneumonia was identified ($p = 0.06$). Given the similarities in the incidences of stillbirth, low 5-minute Apgar scores, and death from sepsis, a sample size of at least 1400 patients would be required to demonstrate a significant reduction ($\alpha = 0.05$, power = 0.80). A significantly larger sample size would be required to show a reduction in other morbidities.

In an attempt to identify a subgroup of women who might benefit more from antibiotic treatment, we assessed latency on the basis of the development of maternal infection at delivery. As depicted in Fig. 2, a statistically significant difference in latency was identified only in the population destined to have chorioamnionitis. It is interesting that the incidence of both clinical infection and suspected infection was not decreased with antibiotic therapy, but their onset was simply delayed.

Amniocentesis was performed when a 1 × 1 cm, cord-free pocket of amniotic fluid could be identified. With this policy, amniotic fluid was obtained in 45% of patients and there were no identified complications. Women with positive amniotic fluid cultures had short latencies and generally were delivered before the culture results became available. Two women in the placebo group with prolonged latencies (12 and 16 days) had enterococci grown from the amniotic fluid. Thus oral erythromycin was not effective in delaying delivery in the group of women with positive amniotic fluid cultures. The group with adequate fluid and negative cultures appeared to be at low risk for infection and therefore did not benefit from antibiotic therapy. Surprisingly, a significant delay in delivery was seen in the population with initial oligohydramnios precluding amniocentesis. It has been previously postulated that oligohydramnios is associated with an increased risk of subsequent clinical infection.[13, 14] The findings of this study indicate that this subgroup of patients might benefit from the use of prophylactic antibiotic therapy.

Morales et al.[10] found that the use of ampicillin therapy in patients with premature rupture of the membranes was effective in prolonging latency period only in those women colonized by group B streptococci. Evaluation of our patients revealed similar trends toward prolongation of latency period regardless of colonization with either group B streptococci or *C. trachomatis*. In addition, no prolongation was seen in women with positive cervical *N. gonorrhoeae* cultures. However, because of the small sample size within these subpopulations, statistical inferences cannot be made.

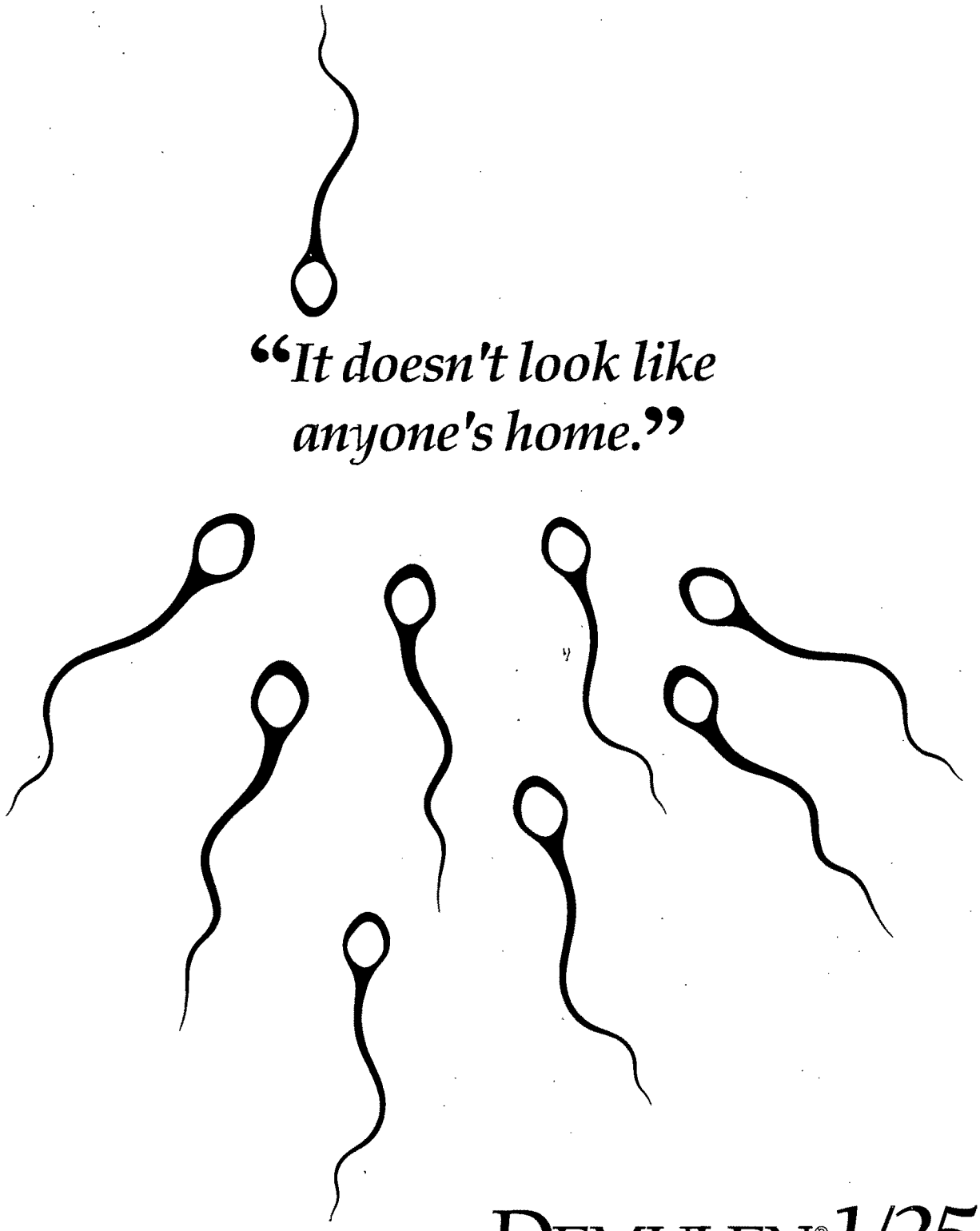
In this study we have observed a wide variety of organisms in the amniotic fluid collected at amniocentesis. Ascending infection with deciduitis and subsequent chorioamnionitis has been postulated with preterm premature rupture of the membranes[15, 16]; therefore it would be reasonable to target antibiotic therapy at these organisms. Erythromycin enjoys rapid oral absorption and has good pelvic soft tissue penetration.[17, 18] In addition, it is effective against a variety of gram-positive and gram-negative organisms, *C. trachomatis*, and *Ureaplasma urealyticum*.[19] On the other hand, ampicillin and clindamycin are broad-spectrum agents with overlapping and antimicrobial activity complementary to erythromycin.[20] Thus combinations of these three agents, in oral or parenteral form, or single agents with broader-spectrum coverage might be more effective in treating the organisms identified in the amniotic fluid of patients studied.

In summary, the use of prophylactic oral erythromycin therapy in patients with preterm premature rupture of the membranes resulted in significant prolongation of latency period. However, this therapy was not associated with any reduction in either maternal or neonatal morbidity.

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BRIEF SUMMARY

Demulen® 1/35: Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients. **Demulen® 1/50:** Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients.

Indication and usage: For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published.

Dose-related Thromboembolic Risk: Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen.

Contraindications: Thrombophlebitis; thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products.

Warnings:

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 15-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <30 mcg estrogen, vs 13 with higher doses. Latter group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myocardial ischemia, occurs with high progestational activity. Consider amount of both steroids in choosing OC. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

Be alert to earliest manifestations of TE disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. 2. *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer with combination or progestogen-only OCs. Studies show no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose, and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors and in various subgroups. Decrease in benign breast tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, CIN, erosion, and carcinoma in long-term users. Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. 4. *Hepatic adenomas, etc.* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur with OC use. 5. *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES-exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,000 births, compared to 7.8 for those unexposed (P<.05). Female hormone exposure in utero was associated with testicular and ovarian cancer. More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, stop OC and apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternative contraceptive for 3 months before attempting to conceive, though this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy tests. 6. *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. Bile duct cancer has been reported. 7. *Carbohydrate and lipid metabolism:* Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides, total phospholipids, and lipoproteins may increase. 8. *Elevated blood pressure* (BP) occurs in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but increases with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pregnancy; familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. 9. *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. 10. *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a higher-estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. 11. *Ectopics:* In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. 12. *Breast feeding:* OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. 13. *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T_4); decreased T_3 uptake, unaltered free T_4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydial and gonorrheal prevalence is increased; do not assume OC protects against chlamydial PID. HIV seropositivity was associated with OC use. *Information for patients.* See patient labeling. *Drug interactions.* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hidradenitis suppurativa, hemolytic uremia, malignant hypertension, impaired renal function, acute renal failure, sometimes irreversible, itching, vaginitis, porphyria, PVCs, abnormal EKG, pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis. Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use and is available on request.**

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Oral folic acid supplementation for cervical dysplasia: A clinical intervention trial

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OBJECTIVE: We attempted to evaluate the effect of oral folic acid supplementation on the course of cervical dysplasia.

STUDY DESIGN: A total of 235 subjects with grade 1 or 2 cervical intraepithelial neoplasia were randomly assigned to receive either 10 mg of folic acid or a placebo daily for 6 months. Clinical status, human papillomavirus type 16 infection, and blood folate levels were monitored at 2-month intervals. Outcome data were subjected to χ^2 analysis.

RESULTS: The prevalence of human papillomavirus type 16 infection initially was 16% among subjects in the upper tertile of red blood cell folate versus 37% in the lower tertile (trend $p = 0.035$). After 6 months no significant differences were observed between supplemented and unsupplemented subjects regarding dysplasia status, biopsy results, or prevalence of human papillomavirus type 16 infection.

CONCLUSION: Folate deficiency may be involved as a cocarcinogen during the initiation of cervical dysplasia, but folic acid supplements do not alter the course of established disease. (AM J OBSTET GYNECOL 1992;166:803-9.)

Key words: Cancer, cervical dysplasia, nutrition, folate, papillomavirus

There is evidence that folate deficiency may be involved in the etiology of cancer of the cervix,[1, 2] esophagus,[3] colon,[4] bronchi,[5] and hematopoietic system of humans,[6] as well as in certain experimental models of carcinogenesis.[7] Considerable interest has developed in cervical dysplasia (cervical intraepithelial neoplasia and squamous intraepithelial lesions), not only because of its high incidence but also because of a possible relationship to oral contraceptive agents,[8] which are reported to lower circulating folate levels[9] and to modify cervical cytologic characteristics.[1, 10] Recent evidence[10a] has shown that folate nutritional status interacts strongly with several established risk factors for cervical intraepithelial neoplasia, such as cigarette smoking, infection with human papillomavirus (HPV), parity, and oral contraceptive use. Normal levels of folate appear to protect against cervical intraepithelial neoplasia and to minimize the risks ordinarily associated with these factors. Cigarette smoking, pregnancy, and oral contraceptive use may be associated with depressed circulating levels of folate.

The role of papillomavirus infection as an etiologic factor in cervical dysplasia remains perplexing. Although HPV genomes can be demonstrated in a high proportion of cervical cancers,[11] they are also demonstrable in cervicovaginal cells from normal women[12] and in normal tissue adjacent to neoplastic lesions.[13] For these reasons it is necessary to consider whether the oncogenic potential of HPV genomes is expressed or remains latent.

In 1982 Butterworth et al.[1] reported that a 3-month period of daily oral folic acid supplementation in 22 subjects was associated with improvement in the cytologic manifestations of dysplasia and with less severe biopsy findings when compared with those of 25 placebo-treated control subjects. The study was undertaken because of morphologic similarities between the cytologic features of megaloblastosis seen with folate deficiency and the changes associated with dysplasia.[1] Megaloblastic features are "deceptively similar" to those observed in early cervical cancer.[14] The report by Butterworth et al.[1] suggested that localized folate deficiency, which is sometimes misdiagnosed as cervical dysplasia, could be a component of the dysplastic process. Subsequently, a larger and more comprehensive study with two major parts was organized by Butterworth et al. (unpublished data). In the first phase of the project a case-control study that was based on 464 subjects derived from screening of 726 candidates was

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reported.[10a] Women who qualified were invited to participate in the second phase, a randomized, placebo-controlled, double-blind clinical intervention trial that required three additional visits at 2-month intervals. We present the findings in 235 subjects who were randomized to receive either the folic acid supplement or placebo.

Material and methods

This project was reviewed, approved, and monitored annually by the Institutional Review Board of the University of Alabama at Birmingham. Informed consent was given by all participants. All subjects were women attending a county health department clinic, usually for family planning, although other reasons included postpartum examination and routine health evaluation. The original examination and Papanicolaou smear collection were performed by a nurse-practitioner. Subjects with abnormal findings were referred to the central colposcopy clinic operated by the Department of Obstetrics and Gynecology of the University of Alabama at Birmingham in conjunction with the county health department. A physician gave each subject a gynecologic examination that included colposcopy, a routine Papanicolaou test, a herpes culture, a wet smear for vaginal pathogens such as trichomonas, chlamydia, and candida, when indicated, and a cervicovaginal scraping to test for HPV-16. Lesions were diagramed and photographed. The squamocolumnar transition zone was identified. Punch biopsy was deferred. A confidential questionnaire to obtain the gynecologic history, educational background, demographic data, socioeconomic status, and personal habits of each participant was administered by a registered nurse.

A case was defined as a woman with a combination of two abnormalities: at least one abnormal Papanicolaou smear showing mild or moderate cervical dysplasia (grade 1 or 2 cervical intraepithelial neoplasia) and a lesion with features of dysplasia visible by colposcopy. All Papanicolaou smears and all biopsy specimens were evaluated by the same cytopathologist. The degree of severity of disease at the time of entry into the study was determined by the most advanced reading of any available smear. The cervical intraepithelial neoplasia classification was based on criteria described by Ferenczy and Winkler.[15] With respect to the Bethesda system grade 1 cervical intraepithelial neoplasia corresponds to "low-grade squamous intraepithelial lesion with mild dysplasia" and grade 2 and 3 cervical intraepithelial neoplasia corresponds to "high-grade squamous intraepithelial lesion and moderate or severe dysplasia." An additional category consisted of women with lesions visible with colposcopy but with cytologic features, such as nuclear enlargement and irregularity, that are not diagnostic of dysplasia. Patients with these lesions were classified as having "atypia less than dysplasia"; they were not included in the current series of cases and have been described separately.[16]

Accrual of patients and data began in November 1985 and extended through March 1990. After the interview, evaluation, and collection of baseline data, women who qualified as cases were invited to enter the vitamin supplement trial and to return for three additional follow-up examinations at 2-month intervals. The risks and benefits of study participation were carefully explained, and the option of conventional management at any time (including local surgical procedures such as cryosurgery or laser beam surgery) was provided. Colposcopic and cytologic examination was performed at each visit. In addition, a punch biopsy was performed under direct vision at the final visit. Subjects received a 2-month supply of capsules that appeared identical but contained either 10 mg of folic acid (pteroylglutamic acid, United States Pharmacopeia) or 10 mg of ascorbic acid (United States Pharmacopeia) as the placebo. Ascorbic acid was selected as a placebo because it would not be likely to have a therapeutic effect in an apparently healthy population at this level of intake. Allocation to a treatment group was made on the basis of a computer-generated list of random numbers maintained by the pharmacy staff, so that all persons involved in patient management were unaware of treatment status. Prescription refills were in accord with the prior assignment.

Laboratory procedures. Blood specimens (taken with the patient in a nonfasting state) were collected at each visit, protected from light, and processed within 3 hours. Processed samples were stored in aliquots at −70° C for analysis at a later time, usually within a few days. Standard quality-control procedures were used during the study to ensure reproducibility. All procedures for biochemical assessment of nutritional status followed standard methods as described by Butterworth et al.[10a] Folate assays in plasma and red blood cells used *Lactobacillus casei* as the test organism.

HPV detection was performed with the Southern blot deoxyribonucleic acid (DNA) hybridization method[16, 17] on cells obtained by cervicovaginal scraping at the time of colposcopy at the first visit and at the final visit. HPV-16 DNA cloned in pBR322 was generously provided by Dr. Harald zur Hausen. Each blot contained an extract of the cervical carcinoma cell line CaSki (American Type Culture Collection) as its HPV-16 positive marker, an extract of normal placenta as the negative marker, and appropriate markers to indicate molecular size.

All results were computerized, stored, and processed in a single centralized location. Information regarding demographic characteristics, clinical and nutritional assessments, treatment, and follow-up status was recorded on specially designed case report forms to facilitate computerization and processing. Quality-con-

trol procedures and a feedback system were instituted to ensure the accuracy and completeness of the data collected. The χ^2 test was used to test the homogeneity of the treatment groups with respect to baseline characteristics. The χ^2 test was also used to evaluate the overall efficacy of the two treatments by comparison of the results of Papanicolaou smears and biopsy. Further comparison of the outcome of the treatments with covariate adjustments was performed with the "polytomous" logistic regression procedure.[18]

Results

A comparison of demographic characteristics, personal habits, sexual and reproductive history, and biochemical indices of nutritional status revealed that the placebo-treated group and the folic acid–supplemented group were suitably similar for statistical analysis. The population was from a lower socioeconomic group (mean annual family income $7740) and was 64% black and 36% white. The mean age was 25.0 years (14% <20 and 2% >40), and median level of formal education was 11.7 years. There was no significant difference between treatment categories with regard to age at first intercourse, parity, smoking habits, oral contraceptive use (80% current users), and rate of HPV-16 infection (30% positive). The single exception was that 46% of folic acid–supplemented subjects reported more than four sexual partners, compared with 32% in the placebo category ($p = 0.006$). A suitable adjustment was made for this difference in subsequent analyses. These laboratory indices at the initial visit were similar in the placebo-treated group and the folic acid–supplemented group: hematocrit, vitamin A (plasma retinol), plasma carotenoids, plasma ascorbate, plasma vitamin B_{12}, plasma folate, red blood cell folate, plasma zinc, red blood cell zinc, plasma copper, and the deoxyuridine suppression test.

An indication of compliance with the protocol may be derived from the number of subjects who returned for scheduled follow-up visits. Of 121 subjects assigned to receive folic acid, 108, 104, and 102 returned for appointments at 2, 4, and 6 months, respectively. All had colposcopic and cytologic evaluations at each visit, and 91 had a punch biopsy performed at the last visit; a biopsy was not considered necessary in 11 subjects. Of 114 subjects assigned to receive placebo, 98, 96, and 97 returned for appointments at 2, 4, and 6 months, respectively. They also had colposcopic and cytologic evaluations at each visit, and 86 had a punch biopsy at the last visit; a biopsy was not considered necessary in 11 subjects. Two hundred thirty-five subjects enrolled at the initial visit; of this number, 199 completed the 6-month evaluation. The dropout rate was 15.3%.

Initial red blood cell folate level (mean ± SE) for the subjects subsequently assigned to receive folic acid was 224 ± 32 ng/ml; the corresponding value for subjects assigned to receive the placebo was 234 ± 12 ng/ml. After 6 months of supplementation the red blood cell folate level was 753 ± 50 ng/ml in those who received the active material. The value remained essentially unchanged (254 ± 19) in the placebo group. Plasma ascorbate levels were not significantly affected by the placebo; they were 5.3 ± 0.3 ng/ml at the outset and 4.7 ± 0.4 ng/ml at the end of the trial period.

Fig. 1 displays the cumulative percentage changes in the red blood cell folate level of all subjects between initial and final visits according to treatment category (folic acid or placebo). A high degree of compliance is seen in the distribution pattern. Values in approximately 80% of the individuals assigned to receive placebo clustered around a zero change, and only eight subjects had an increase of >100% in red blood cell folate levels. It is not known if these subjects were taking self-administered supplements or if they had received active capsules in error. Subsequent analyses included comparison by levels of red blood cell folate and not merely by assignment to a category. In contrast, approximately 70% of the individuals assigned to receive active folic acid showed an increase of >100% in red blood cell folate levels; values in nine subjects increased ≥700% from baseline values.

Fig. 2 graphically illustrates the changes in Papanicolaou smear results from the initial visit to the last and the biopsy result according to treatment groups. No significant differences were observed for either outcome between subjects who had received folic acid and those who had received placebo. In the multivariate logistic regression analysis, initial Papanicolaou smear, race, and number of sexual partners were found to correlate significantly with the biopsy results. After adjustments for these three factors, the difference in biopsy results between the two treatment groups remains not statistically significant ($p = 0.833$). Although each subject had a visible colposcopic lesion and an abnormal Papanicolaou smear initially, >60% in each treatment group had normal biopsy specimens at the end of 6 months. The supplement was not accompanied by any evidence of worsening with regard to cytologic or biopsy results.

Table I presents the changes observed in HPV status of cervicovaginal scrapings between the initial visit and the final follow-up examination according to levels of red blood cell folate. A trend showing significantly more positive tests for HPV-16 was observed initially with lower levels of red blood cell folate ($p = 0.035$). The same basic patterns persisted after the 6-month period of supplementation, although the trend became only marginally significant ($p = 0.071$). There was a decline in lower values and an increase in higher values of red blood cell folate in the population as a result of changes in the supplemented group. Table I also pre-

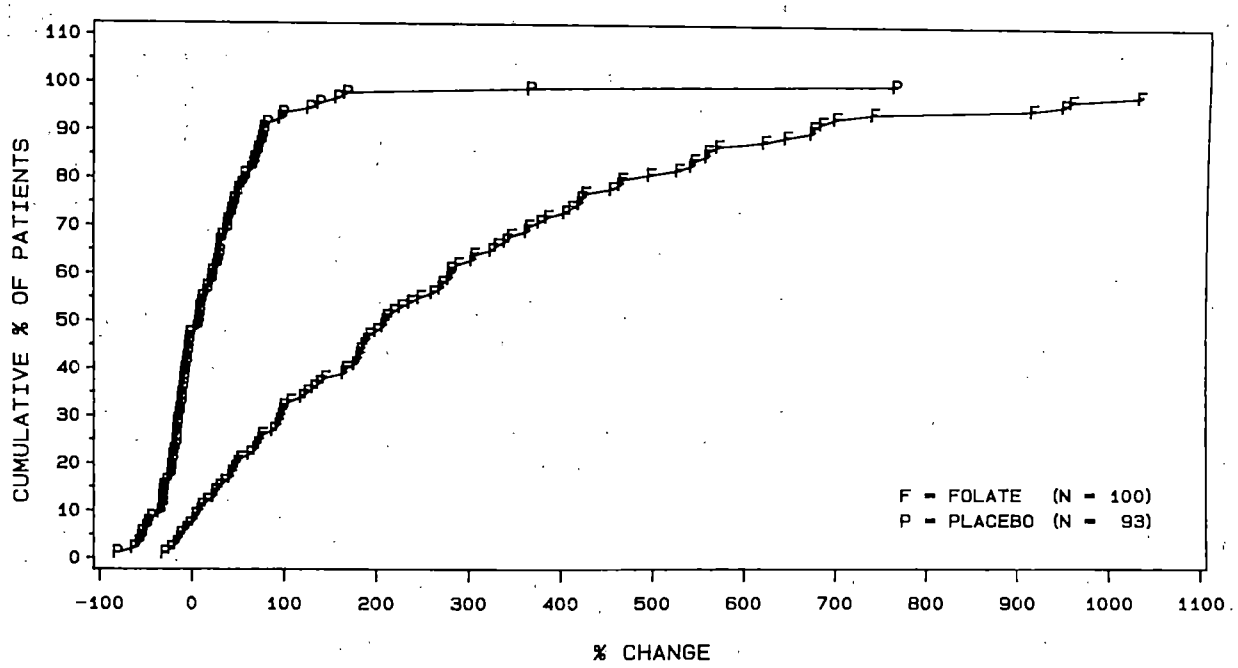


Fig. 1. Cumulative percentages of change over baseline in red blood cell folate concentration for subjects receiving folic acid supplement *(F)* and placebo *(P)*. Patterns clearly define separate populations and indicate high degree of compliance. It is possible that two individuals showing a high percentage of change in placebo group received active medication in error. Analysis of outcome included red blood cell folate status and was not limited to assigned category (see text).

Table I. Initial and final HPV-16 test results and number of positive biopsy findings (grades 1, 2, and 3 cervical intraepithelial neoplasia) according to upper, middle, and lower tertiles of red blood cell folate

	Red blood cell folate		
	≥290 ng/ml	*180-289 ng/ml*	*<180 ng/ml*
Initial HPV-16 results			
Positive			
No.	8	25	34
%	16	32	37
Total No.	49	79	91
p Value			0.035
Final HPV-16 results			
Positive			
No.	18	14	13
%	20	39	32
Total No.	90	36	41
p Value			0.071
Final biopsy results			
Cervical intraepithelial neoplasia present			
No.	30	13	15
%	32	36	38
Total No.	95	36	39
p Value			0.378

The distribution of HPV-16–positive cases among all subjects according to three categories of red blood cell folate at the time of the initial evaluation *(top)* and again after the 6-month observation period *(middle)*. Although supplementation nearly doubled the number of individuals in the high folate category, the same basic trend persisted, but at a less significant level ($p = 0.071$). Also shown *(bottom)* is the distribution of positive biopsy findings (grades 1, 2, and 3 cervical intraepithelial neoplasia) according to categories of red blood cell folate for all subjects. The percentage of subjects with a positive biopsy finding was slightly greater with lower red blood cell folate levels, but the difference was not statistically significant.

sents the relationship of the biopsy classification to the final red blood cell folate status. All subjects who underwent biopsy are included whether they were assigned to receive the folic acid supplement or placebo. There were slightly more positive findings (i.e., grades 1, 2, and 3 cervical intraepithelial neoplasia) among subjects with lower red blood cell folate levels, but the difference was not statistically significant. Tabulation

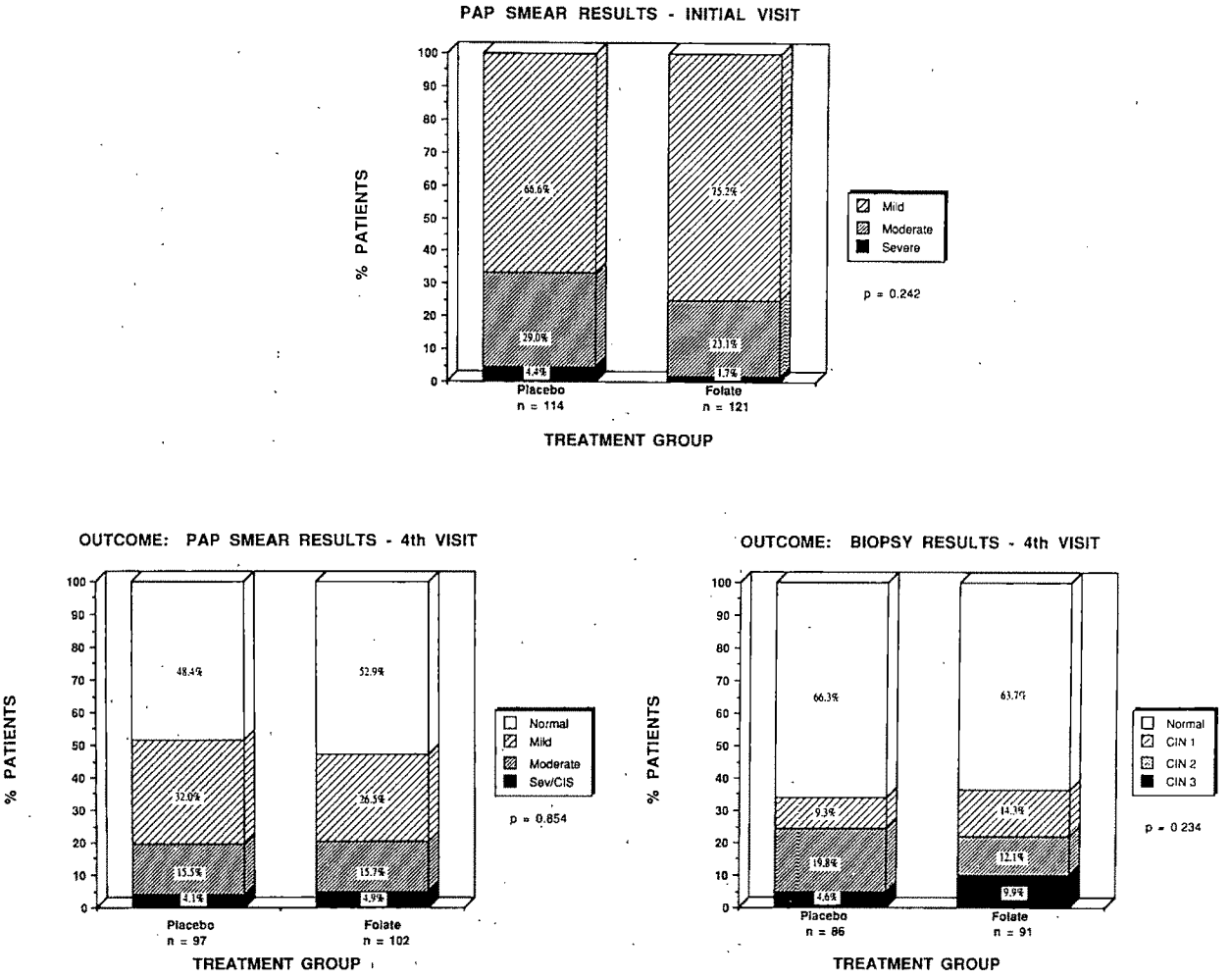


Fig. 2. *Upper panel* indicates similarity in distribution of cases according to severity of cytologic lesions for subjects randomly assigned to receive either placebo or folic acid supplementation. Only subjects with an abnormal Papanicolaou smear were entered into clinical trial. *Lower left panel,* Distribution of Papanicolaou smear results for two categories after 6 months of observation. There is no significant difference between outcome in placebo or folic acid–supplemented subjects ($p = 0.854$). *Lower right panel,* Biopsy results for two categories. There is no significant difference between population receiving placebo and those who received folic acid supplement ($p = 0.234$). After 6 months of observation approximately half of the entering subjects were normal according to Papanicolaou smear results and two thirds were normal according to biopsy results, regardless of treatment category.

of the same biopsy results according to the initial red blood cell folate levels (not shown) reveals essentially the same distribution pattern, with 31%, 33%, and 39% in the upper, middle, and lower tertiles, respectively.

Comment

This study was designed to test the hypothesis that high-dose oral supplements of folic acid will modify the course of cervical dysplasia and improve its cytologic and histologic manifestations. The findings reject the hypothesis but provide useful information that was not anticipated at the outset. At the time the project was planned, it was believed that a localized folate deficiency may exist in the cervix without evidence of systemic depletion. Also, the role of papillomaviruses was gaining recognition as an etiologic factor of cervical dysplasia, although this role was not well established. Our results, as well as those of a case-control study,[10a] indicate a higher prevalence of dysplasia associated with HPV-16 infection among women in the lower two tertiles of red blood cell folate than in the highest tertile. However, oral folic acid supplementation given over a period of 6 months appears to have no significant effects on the course of established cases of dysplasia. Possible explanations for the difference between the current findings and the earlier report[1] include a more adequate sample size, exclusion of patients classified as having atypia less than dysplasia, and a longer period of observation. We entertained the possibility that the 10 mg dose of ascorbate, which is generally regarded as trivial, might have had a therapeutic effect. However, this seems unlikely because plasma ascorbate levels

were not associated with dysplasia[10a] and they did not change in the subjects receiving placebo during the period of observation. Zeigler et al.[19] also found no significant differences in cervical cancer incidence associated with quartiles of ascorbate intake ranging from <93 mg/day to >212 mg/day.

The high rate of apparent regression in both the placebo-treated and folic acid–supplemented groups of our intervention trial (66.3% and 63.7%, respectively) may have contributed to the lack of significant differences. Although there are conflicting reports regarding rates of spontaneous regression, Nasiell et al.[20] observed a 62% rate of regression to normal among 555 women with mild dysplasia who underwent follow-up for an average of 39 months without major treatment.

In a review of the role of vitamin B_{12} and folate deficiencies in carcinogenesis, Eto and Krumdieck[21] observed that neither deficiency is carcinogenic by itself but that each may increase susceptibility to the action of other carcinogens. Folate deficiency is associated with chromosome breaks,[22] which can allow the insertion of viral oncogenes or can cause translocations with a resultant alteration in genetic regulatory mechanisms.[23] A deficiency of folate and related methyl donors has also been reported to enhance the expression of endogenous and exogenous oncogenes that might otherwise by suppressed.[7]

According to the concept of multiple stages in the process of carcinogenesis, the initiation phase of cervical intraepithelial neoplasia may begin when a genome of oncogenic HPV is incorporated into the host cell DNA. Nearly every human chromosome carries at least one protooncogene, and human tumor cells commonly have mutations that permit either the activation of oncogenes or the inactivation of tumor suppressor genes. Therefore enhanced expression of genetic information in the presence of sublethal folate deficiency or rearrangement of genetic sequences that result from folate-related chromosome breaks could initiate uncontrolled cell growth. There is evidence that in an established line of cultured cervical cancer cells the HPV-18 genome is present at a constant location on chromosome 12 and that insertion may have been facilitated by a folate-sensitive fragile site at the same location.[24] Thus folate deficiency could be a key factor in the initiation of cancer by an oncogenic strain of HPV.

In keeping with a study that compared these same cases of dysplasia with controls,[10a] the lowest rate of HPV-16 infection was observed in the group with the highest levels of red blood cell folate. A plausible explanation for these findings is that folate deficiency serves as a cocarcinogen in the presence of exposure to an oncogenic strain of HPV. After a viral genome has been incorporated into the host DNA, folic acid supplements have little or no effect on the course of the infection.

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Efficacy of the fetal-pelvic index in nulliparous women at high risk for fetal-pelvic disproportion

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OBJECTIVE: The null hypothesis of this study is that the fetal-pelvic index will not determine the presence or absence of fetal-pelvic disproportion in 137 nulliparous women at high risk for fetal-pelvic disproportion.
STUDY DESIGN: This study was undertaken by comparing the blinded fetal-pelvic index values and two other methods of identifying fetal-pelvic disproportion, ultrasonography-derived estimated fetal weight ≥4000 gm and Mengert's index, to delivery outcomes in 137 nulliparous women at high risk for fetal-pelvic disproportion.
RESULTS: After adequate labor trials, 55 of 73 patients who required operative intervention had a positive fetal-pelvic index (sensitivity = 75%). Seventeen of the 18 patients with a false-negative fetal-pelvic index value had persistent malpositions (15 occipitoposterior, 1 occipitotransverse, and 1 face presentation). Spontaneous vaginal deliveries occurred in 64 patients with 62 of them having a negative fetal-pelvic index (specificity 97%). The overall predictability of the fetal-pelvic index in this patient population was 85% and the positive predictability was 96%. However, neither ultrasonography-derived estimated fetal weight nor x-ray pelvimetry, when used alone, accurately detected the presence or absence of fetal-pelvic disproportion in nulliparous women at high risk for the condition.
CONCLUSION: The fetal-pelvic index is efficacious in determining the presence or absence of fetal-pelvic disproportion in nulliparous women at high risk for the condition. (Am J Obstet Gynecol 1992;166:810-4.)

Key words: Fetal-pelvic disproportion, fetal-pelvic index, nullipara, labor

The laboring nulliparous patient presents a unique situation to the obstetrician because her pelvis (passage) and her fetus (passenger) are about to have their first confrontation. The circumstance in which the fetus is too large to pass through the pelvis is defined as fetal-pelvic disproportion. Furthermore, for those patients in whom fetal-pelvic disproportion is present, maternal and perinatal morbidity and mortality rates are increased.[1-9]

Numerous methods to identify fetal-pelvic disproportion have been tested, including a clinical estimation of both the pelvis size and fetal weight, maternal radiographic pelvimetry,[10,11] ultrasonography-derived estimated fetal weight,[12] and simply a trial of labor for all cephalic presentations.[13] Although each of these methods has demonstrated limited success, none has proved reliable in prospectively diagnosing fetal-pelvic disproportion.

The fetal-pelvic index was introduced as a method of identifying both the presence and absence of fetal-pelvic disproportion, by comparing the respective fetal head and abdominal circumferences with the maternal inlet and midpelvic circumferences.[14] Although the fetal-pelvic index has proved to be a reliable method of determining fetal-pelvic disproportion in patients delivered of macrosomic neonates,[15] in those requiring labor inductions,[16] in those with abnormal labor patterns requiring augmentation,[17] and in those attempting vaginal birth after previous cesarean section,[18] the efficacy of the fetal-pelvic index in nulliparous patients at high risk for fetal-pelvic disproportion has not been determined. The objective of this study is to compare the efficacy of three methods used to identify fetal-pelvic disproportion, ultrasonography-derived estimated fetal weight ≥4000 gm, Mengert's index, and the fetal-pelvic index, in nulliparous women at high risk for fetal-pelvic disproportion.

Material and methods

One hundred sixty-four nulliparous women who were seen by the Oklahoma Memorial Hospital labor and delivery personnel at term gestation with either an unengaged fetal presentation, clinically suspected fetal macrosomia, a small maternal pelvis, or an indication for labor induction or augmentation were entered into this study (Table I). Twenty-seven patients were excluded from data analysis after undergoing an operative delivery before an adequate labor trial (four with suspected macrosomia—no labor trial, three positive

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and one negative fetal-pelvic indexes; six with failed induction and cervical dilatation <4 cm, one positive and five negative fetal-pelvic indexes; four with breech presentation and no labor, three positive and one negative fetal-pelvic indexes; and one with persistent occipitoposterior presentation with fetal-pelvic index used in management) or because of fetal distress (12 patients with four positive and eight negative fetal-pelvic indexes). The remaining 137 patients had an adequate trial of labor, defined as uterine contractions of a frequency of at least three contractions in 10 minutes, an intensity of >50 mm Hg, and a duration of >60 seconds by an intrauterine pressure catheter and a cervical dilatation of ≥5 cm for a minimum of 2 hours. Spontaneous labor occurred in 55 patients, and 82 patients required oxytocin to stimulate labor (37 inductions, 45 augmentations).

Fetal ultrasonographic evaluations were performed in the labor rooms with a portable linar array ultrasonography machine. Diameters of the fetal head, transverse and anteroposterior, were determined by means of built-in electronic calipers applied to a freeze-frame image of the plane that included the thalamus, septum cavum pellucidum, and a symmetrically aligned midline echo. Diameters of the fetal abdomen, transverse and anteroposterior, were determined by means of built-in electronic calipers applied to a freeze-frame image of the plane perpendicular to the fetal spine at the level of the portal vein bifurcation and stomach.

Maternal pelvic measurements were obtained with the Colcher-Sussman x-ray pelvimetry technique.[11] This method consists of simple patient positioning so that lateral and anteroposterior views of the pelvis are obtained. A ruler is positioned to allow its inclusion on the radiograph for straightforward pelvic diameter measurement. Diameters of the inlet and midpelvis, transverse and anteroposterior, were measured before either the active phase of labor or oxytocin administration.

From the sites of individually measured diameters respective circumferences of the fetal head, fetal abdomen, maternal pelvic inlet, and maternal midpelvis were calculated with the simple equation: Circumference = (Transverse diameter + Anteroposterior diameter) × ½ π. On the basis of the four circumference differences between the fetus and maternal pelvis (head circumference − pelvic inlet circumference, head circumference − midpelvis circumference, abdominal circumference − pelvic inlet circumference, abdominal circumference − midpelvis circumference), a fetal-pelvic index value was derived from the sum of the two most positive circumference differences.[14] A positive fetal-pelvic index value indicates the fetus to be larger than the maternal pelvis, and a negative fetal-pelvic index value, the fetus to be smaller than the maternal pelvis. It is our hypothesis that a positive or negative fetal-pelvic index value indicates the presence or absence of fetal-pelvic disproportion, respectively. Results of the fetal-pelvic index values were blinded from the clinicians managing the patient's labor, and delivery outcome results were blinded from the investigators.

Table I. Patient population

Patient category	*No.*
Unengaged fetal presentation	34
Suspected small maternal pelvis	14
Suspected fetal macrosomia (>4000 gm)	7
Indications for induction	
Premature rupture of membranes	11
Postdates	9
Preeclampsia	9
Diabetes mellitus	5
Other	3
Labor pattern for augmentation	
Primary dysfunctional	12
Secondary arrest	20
Combined	13

The fetal-pelvic index and two other methods of identifying fetal-pelvic disproportion were compared with regard to both clinical outcome and the accuracy of predicting the presence or absence of fetal-pelvic disproportion. The two methods were ultrasonography-derived estimated fetal weights (tables of Shepard et al.[12]) with an estimated fetal weight of ≥4000 gm as the threshold weight for identifying the presence of fetal-pelvic disproportion and Mengert's index[10] of maternal pelvic area capacity with a contracted pelvis defined as <85% of average pelvic capacity, <123 cm^2 for the inlet or <106 cm^2 for the midpelvis.

This study was reviewed and approved by the institutional review board. Continuous data were reported as the mean ± SD and were analyzed with an independent two-tailed t test with categoric data being analyzed by χ^2 and Fisher exact test where appropriate. A p value of <0.05 was considered significant. An operative delivery was defined as either an operative vaginal delivery, in patients requiring a midpelvic instrumental delivery or an extra maneuver to reduce a shoulder dystocia and achieve delivery, or a cesarean section for the clinical diagnosis of failure to progress.

Results

After an adequate labor trial, 73 patients required operative intervention, 65 cesarean section and eight operative vaginal, to achieve delivery. Sixty-four patients had spontaneous vaginal deliveries. The mean maternal age and maximum dose of oxytocin were comparable between the groups (Table II). The frequency of those having spontaneous labor and those requiring oxytocin induction or augmentation of labor were similar between the groups. However, the mean gestational

Table II. Results of descriptive variables

Delivery outcome	No.	Age (yr)	Gestational* age (wk)	Type of labor: Spontaneous (No.)	Oxytocin: Induction: No.	Oxytocin: Induction: Maximum dose (mU/min)	Oxytocin: Augmentation: No.	Oxytocin: Augmentation: Maximum dose (mU/min)	Birth weight† (gm)
Operative delivery	73	21 ± 4	40.5 ± 1.8	29	18	11 ± 6	26	7 ± 7	3705 ± 485
Spontaneous vaginal	64	20 ± 4	39.8 ± 1.7	26	19	10 ± 5	19	7 ± 6	3372 ± 428

*$p < 0.025$.
†$p < 0.001$.

Table III. Results of measurement data

Delivery outcome	Ultrasonography (cm): No.	Ultrasonography (cm): Head circumference	Ultrasonography (cm): Abdominal circumference	Radiographic pelvimetry (cm): Inlet circumference	Radiographic pelvimetry (cm): Midpelvic circumference
Operative delivery	73	33.4 ± 1.2	35.6 ± 2.3	38.6 ± 2.3	34.0 ± 2.2
Spontaneous vaginal delivery	64	33.1 ± 1.1	34.0 ± 1.8	39.5 ± 2.1	35.7 ± 2.0
p Value		NS	<0.001	<0.025	<0.001

NS, Not significant.

age at delivery and birth weight were significantly greater in the operative delivery group compared with the spontaneous vaginal delivery group (Table II).

Although the mean fetal head circumferences were similar between the groups, the mean fetal abdominal circumferences were significantly larger in the operative delivery group (Table III). Interestingly, the mean maternal pelvic inlet and midpelvic circumferences in the operative delivery group were significantly smaller than those in the spontaneous vaginal delivery group. There was no classic cephalopelvic disproportion observed because the head circumferences between these two groups were similar and smaller than their respective maternal pelvic circumference measurements. On the basis of these measurement data, fetal-pelvic disproportion exists in the operative delivery group. The fetal measurement that appears to be indicative of fetal-pelvic disproportion is the fetal abdominal circumference, because this measurement in the operative delivery group was larger than its respective midpelvic circumference. However, the fetal abdominal circumference in the spontaneous vaginal delivery group was smaller than its respective maternal midpelvic circumference (Table III).

Of the 73 patients requiring operative intervention to achieve delivery, 55 had a positive fetal-pelvic index value, 50 cesarean and five operative vaginal deliveries, and 18 patients had negative fetal-pelvic index values for a sensitivity of 75% (Table IV). Of the five patients with a positive fetal-pelvic index who required operative vaginal deliveries, two patients had midforceps rotations, two patients had shoulder dystocias, both requiring McRobert's maneuver and suprapelvic pressure, and one patient had a midforceps rotation and shoulder dystocia requiring Wood's maneuver. The 18 patients with negative fetal-pelvic index values who required operative deliveries warrant further discussion. All 15 of those patients requiring cesarean section had persistent fetal malpositions, 14 occipitoposterior and one mentum posterior face presentation. In addition, two of the three patients who required operative vaginal deliveries with negative fetal-pelvic index values had persistent fetal malpositions, one occipitoposterior and one occipitotransverse. The third patient, who required midforceps delivery, had a dense epidural and maternal fatigue after a prolonged second stage of labor.

Sixty-four patients had spontaneous vaginal deliveries with 62 patients having negative fetal-pelvic index values for a specificity of 97% (Table IV). The two patients who were delivered vaginally with positive fetal-pelvic index values both had abdominal-pelvic circumference differences that contributed to their positive fetal-pelvic index values and one of these patients had outlet forceps performed. Overall, the delivery outcome predictability for the fetal-pelvic index was 85% and the positive predictability was 96%.

When the ultrasonography-derived estimated fetal weight was used alone with ≥4000 gm as the threshold weight for suspicion of fetal-pelvic disproportion, the

Table IV. Results of study data

Delivery outcome	*Ultrasonography-estimated fetal weight*		*Radiographic pelvimetry (Mengert's index)*		*Fetal-pelvic index*	
	≥4000 gm	*<4000 gm*	*Contracted*	*Adequate*	*Positive*	*Negative*
Operative	16	57	22	51	55	18
Cesarean	13	52	19	46	50	15*
Vaginal	3	5	3	5	5†	3‡
Spontaneous vaginal	1	63	3	61	2	62
Sensitivity (%)	22		30		75	
Specificity (%)	98		95		97	
Predictability (%)	58		61		85	

*Persistent occipitoposterior, $n = 14$; mentum posterior face presentation, $n = 1$.

†Midforceps rotation, $n = 2$; shoulder dystocia, $n = 2$; midforceps rotation and shoulder dystocia, $n = 1$.

‡Persistent occipitoposterior, $n = 1$; persistent occipitotransverse, $n = 1$.

sensitivity was 22%, specificity 98%, and the overall predictability 58% (Table IV). With Mengert's index alone, with a contracted pelvis defined as <85% of average pelvic capacity area (<123 cm^2 for the inlet or <106 cm^2 for the midpelvis), the sensitivity was 30%, the specificity was 95%, and the overall predictability was 61% (Table III). Therefore neither of these techniques, when used alone, provided accurate identification of fetal-pelvic disproportion because of their respective low sensitivities.

Comment

The nulliparous patient in labor presents a unique challenge to the obstetrician because a fetus has not previously had to negotiate her pelvis. Friedman and Sachtleben,[1-3, 19] in a number of reports, described dysfunctional labor in the nulliparous patient and observed that fetal-pelvic disproportion was not uncommonly the cause, frequently resulting in prolonged labor trials.[3] The majority of these prolonged labor trials ended with an operative delivery, either midpelvic instrument assisted or cesarean section. The maternal and neonatal outcomes in these nulliparous women with prolonged labor trials as a result of fetal-pelvic disproportion are frequently adverse.[2, 3, 9]

Fetal-pelvic disproportion is not the result of dysfunctional labor but occurs when the passenger (fetus) is larger than its respective passage (maternal pelvis). Initially, radiographic pelvimetry was thought to be predictive of obstetric outcome. However, as the obstetrician began to have a more active role in treatment of dysfunctional labor, it was less useful and its use was discontinued.[13, 20, 21] Our study confirms the inability of x-ray pelvimetry, when used alone, to accurately predict the presence of fetal-pelvic disproportion (Table IV). Similarly, the predictability of an arbitrary ultrasonography-derived estimated fetal weight to detect the presence of fetal-pelvic disproportion, when used alone, is unacceptably low (Table IV).

Because attempts at measuring only the passage (maternal pelvis) have failed, it has been recommended that all patients with a cephalic presentation continue to labor until either vaginal delivery is accomplished or labor arrest is blatantly obvious.[13] With this approach an increase in the number of cesarean sections for dystocia has occurred without a determination of the size of the passage (maternal pelvis) and passenger (fetus). Although the philosophy that all women deserve a labor trial has been embraced with open arms, there still remains an increased rate of maternal postcesarean infectious morbidity[4-7] and an increased frequency of acidotic neonates in women with fetal-pelvic disproportion.[8] Therefore the prospective diagnosis of fetal-pelvic disproportion would seem helpful in these patients to avoid prolonged labor trials, which lead to an operative delivery.

The fetal-pelvic index allows the detection of the presence or absence of fetal-pelvic disproportion in the laboring nulliparous patient with a high degree of predictability (Table IV). The unique innovation of this concept is that the size of the passage (maternal pelvis) and the respective passenger (fetus) are compared rather than there being an exclusive investigation of the maternal pelvic size (passage) or the fetal weight (passenger). The overall predictability of the fetal-pelvic index that we observed in this study was similar to the overall predictabilities of the fetal-pelvic index previously reported in other high-risk patient groups.[14-18] The false-negative fetal-pelvic index values observed in this study, with one exception, were consistently due to the presence of persistent fetal malpositions. This type of malposition, occipitoposterior, has been previously observed to occur in nulliparous patients with dysfunctional labor and frequently requires an operative delivery in spite of oxytocin augmentation of labor.[1, 17]

The sensitivity, specificity, and overall predictability of the fetal-pelvic index of nulliparous women from the previous report of patients delivered of macrosomic neonates[15] were 86%, 100%, and 93%, respectively, and these values were 88%, 100%, and 96%, respectively, from women in this study delivered of macrosomic neonates. Similarly, the sensitivity, specificity, and overall

predictability of the fetal-pelvic index of nulliparous women from the previous report of patients requiring labor induction[16] were comparable to the rates of this study in patients who required labor induction (85%, 100%, and 93% vs 83%, 100%, and 92%). The efficacy of the fetal-pelvic index reported previously in nulliparous women requiring labor augmentation[17] was comparable to that of women in this study who required labor augmentation (sensitivity 73%, specificity 100%, and overall predictability 84% vs 67%, 94%, and 78%, respectively). Data generated from this study confirm our previous observations.[15-17]

Because many nulliparous women at high risk for fetal-pelvic disproportion often have prolonged, unsuccessful labor trials and have potential for morbidity,[1-3] we believe that the use of the fetal-pelvic index can alert the obstetrician of the patient in whom an earlier cesarean section during the labor trial may be beneficial. Conversely, reassurance of a vaginal delivery can be provided to both the obstetrician and the nulliparous women, with a negative fetal-pelvic index and without a fetal malposition. Before cesarean section is performed in nulliparous women without a trial of labor, a randomized trial comparing the maternal and perinatal morbidity of patients with a positive fetal-pelvic index who undergo a cesarean section without a labor trial with that of patients with a positive fetal-pelvic index, blinded from the care giver, who undergo a labor trial is needed. This type of study could verify the performance of a cesarean section without a labor trial in nulliparous women with a positive fetal-pelvic index who are at high risk for fetal-pelvic disproportion.

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Superficial laser vulvectomy

V. Surgical debulking is enhanced by adjuvant systemic interferon

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Skillful laser ablation can remove any volume of human papillomavirus–associated vulvar disease but cannot prevent reactivation of the surrounding latent viral reservoir during postoperative healing. Conversely, interferon and 5-fluorouracil are relatively ineffective as primary therapies in clearing bulky lesions. In this study of 71 assessable patients, topical 5-fluorouracil and systemic interferon injections were used postoperatively. Success rates within the adjuvant 5-fluorouracil and laser alone arms were essentially the same (9 of 18 vs 8 of 20). In contrast, outcome in the interferon group was significantly better than that for the other two arms combined (27 of 33 [82%] vs 17 of 38 [45%]; χ^2 10.31; $p < 0.002$). Moreover, 18 of 21 failures (86%) in the first two arms and 3 of 6 failures (50%) in the interferon arm were "rescued" from the need for a second laser surgical procedure by crossover to either the 1 or 3 MIU interferon regimen. Results from this open-label, randomized clinical trial suggest that even a relatively low dose of recombinant interferon, used in combination with effective surgical debulking, can markedly reduce the risk of postoperative recurrence. (Am J Obstet Gynecol 1992;166:815-20.)

Key words: Human papillomavirus vulvar disease, carbon dioxide laser, adjuvant interferon, topical 5-fluorouracil

Historically refractory condylomas and bowenoid dysplasia were managed by prolonged courses of topical 5-fluorouracil cream, in doses that produced widespread cytotoxicity and consequent epithelial sloughing.[1] However, such regimens were extremely painful and had low patient compliance. With the exception of isolated vaginal condylomas, destruction of macroscopic disease foci is better done with the carbon dioxide laser. However, the use of 5-fluorouracil in noncytotoxic doses has had a recent resurgence in modern clinical practice. Sillman and Sedlis[2] have shown, in patients with renal allografts or other immune deficiencies, that the use of lifelong low-dose 5-fluorouracil is an essential prerequisite to disease control. Krebs[3] extended this concept to the treatment of immunologically competent women, reporting improved success (87% vs 62%) in a group randomized to receive biweekly topical 5-fluorouracil as an adjunct to destructive therapy. However, in a retrospective review of 160 women treated primarily by extended laser ablation, we found that the beneficial effects of adjuvant, once-weekly 5-fluorouracil were limited to women with two or more adverse factors (i.e., cigarette smoking, impaired host immune status, high-risk viral type, high-grade dysplastic change, prolonged refractory clinical course, or failed previous extended laser ablation) (88% vs 56%).[4] Unfortunately, in our hands, low-dose 5-fluorouracil has added almost nothing (93% vs 87%) to the efficacy of destructive therapy in less complicated cases.

Over the last 10 years, various cytokines (compounds that regulate the activity of neighboring cells) have been tested for antiviral or immunomodulatory properties. In 1957, preliminary studies recognized the presence of an inducible, nondialyzable factor that protected eukaryotic cells in tissue culture against viral infection.[5] This factor has since been identified as interferon, a group of relatively species-specific glycoproteins with antiviral, antiproliferative, and immunomodulatory properties. Human interferons are classified into three classes (alpha, beta, and gamma), on the basis of antigenic specificity and cell of origin. Pharmacologic preparations came from two sources: natural (a mixture of predominantly alpha interferons obtained by partial purification of virus-stimulated buffy coat leukocytes) and recombinant (a single species of alpha, beta, or gamma interferons harvested by transfection of *Escherichia coli* colonies with plasmids containing the appropriate human genomic sequences). Biologic activity depends on specific cell surface receptors and is mediated through production of chromographically detectable proteins. Much of the antiviral activity is due to the induction of 2′5′-oligoadenylate synthetase, an

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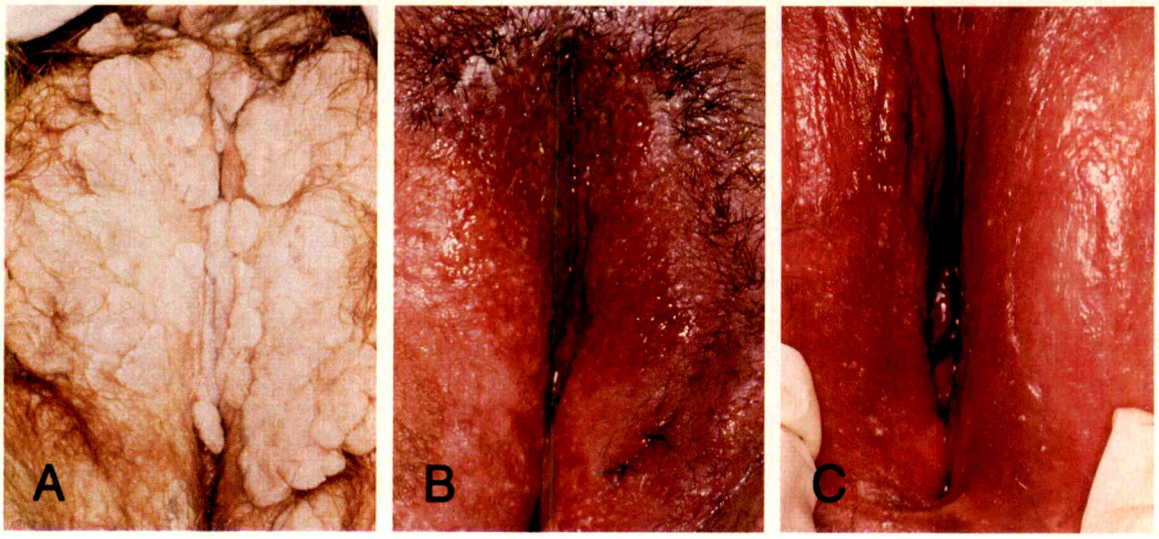


Fig. 1. A, Example of extensive vulvar condylomas, refractory to a variety of office and outpatient therapies over preceding 10-year period. **B,** One week after extended laser ablation, showing speed of healing after such shallow destruction. **C,** Appearances 6 months after laser surgery (and 3 months after discontinuing 10-week interferon course).

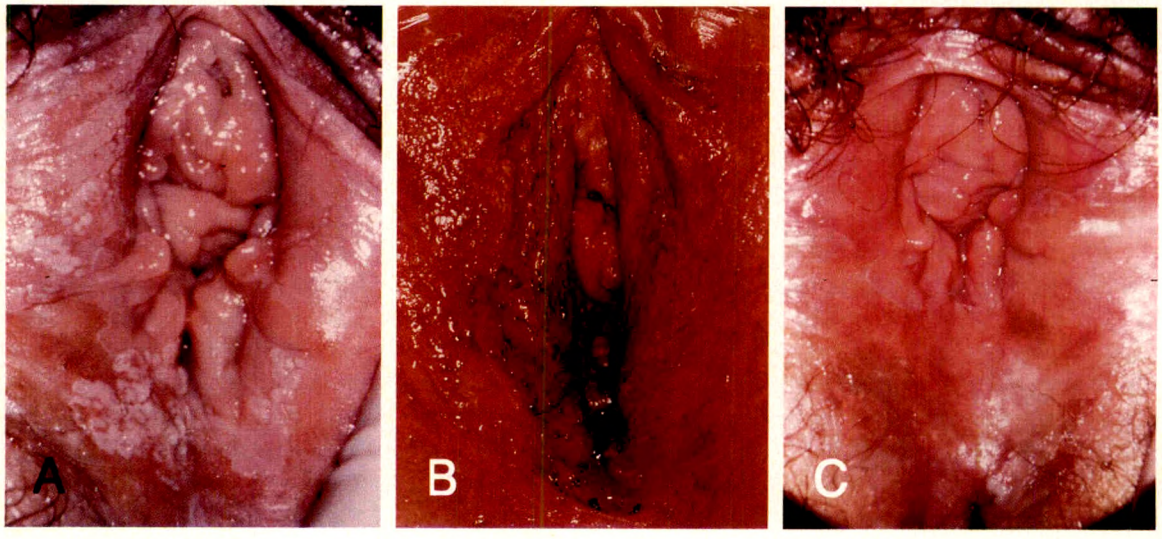


Fig. 2. A, Extensive multifocal vulvar intraepithelial neoplasia, grade 3. **B,** Immediately after extended laser ablation, down to third surgical plane. **C,** Appearances at 6-month follow-up visit.

intracellular enzyme that degrades viral messenger ribonucleic acid, thereby terminating virus replication. Additional antiviral properties are conferred by the induction of protein kinase activity, resulting in the inactivation of cellular factors (such as eukaryotic initiation factor 2) required for the synthesis of viral proteins.[5] Immunomodulatory and antiproliferative properties are mediated through the enhancement of cytotoxic T cells, natural killer cells, antibody-dependent, cell-mediated cytotoxicity, and macrophage activity. Interferon exposure also increases expression of the histocompatibility markers at the cell surface, thereby improving host recognition of virus-infected cells.

All classes of interferon have known activity against papillomaviruses in vivo and in vitro. In tissue culture, natural lymphoblastoid interferon will protect mouse cells against infection with bovine papillomavirus and can also cure infected cells by eliminating the extra chromosomal viral deoxyribonucleic acid.[6] In vivo, clinical experience has confirmed that alpha,[7, 8] beta,[9] and gamma[10] interferons all display definite, albeit unpredictable, activity against expressed human papilloma-

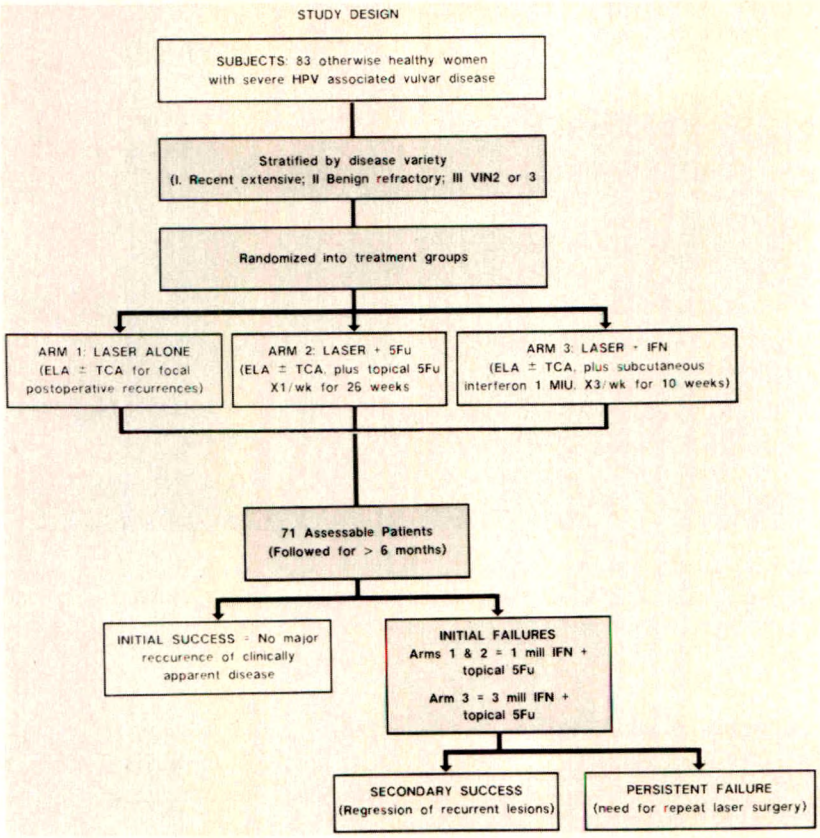


Fig. 3. Diagram depicting study design. *ELA*, Extended laser ablation; *TCA*, 85% trichloracetic acid; *5 FU*, 5-fluorouracil; *IFN*, interferon.

virus infection. Unfortunately, comparison of these studies is impossible because of enormous variations in study design, patient selection, routes of administration, and dosage schedules. Nonetheless, attempts to use interferon as primary therapy have been disappointing,[11] and it is doubtful if the results achieved to date justify the expense, inconvenience, and morbidity of the various regimens used.[12] In this regard, seeking to clear large volumes of virally induced lesions with a drug may be analogous to attempting chemotherapy in advanced ovarian cancer, without prior cytoreductive surgery. This study seeks to evaluate two candidate regimens (5-fluorouracil and interferon) as adjuvants to optimally performed laser surgery in the treatment of confluent, refractory, or dysplastic vulvar disease.

Material and methods

Subjects for this single-center, randomized, open-label trial were adult women with human papillomavirus–associated vulvar disease of sufficient severity to warrant extended laser ablation (Figs. 1 and 2). Patients were recruited from women referred to the private practice of R.R. in Southfield, Michigan, between December 1987 and March 1989. Eligibility reflected three clinical categories: extensive coalescent benign condylomas, condylomas refractory to at least 9 months of simpler therapies, and extensive multifocal vulvar intraepithelial neoplasia (grade 2 or 3). Reasons for exclusion were the presence of a confounding dermatologic disease; a previous invasive malignancy; positive human immunodeficiency virus antibody test; significant renal, hematologic, cardiologic, pulmonary, or hepatic disease; nonavailability for follow-up; or any previous used interferon for any reason. After enrollment, patients were randomized to one of three treatment arms: extended laser ablation alone, extended laser ablation plus once-weekly topical 5-fluorouracil to the vulvar skin for 26 weeks, or extended laser ablation plus 1 MIU recombinant alpha interferon (Roferon A, Hoffman-La Roche, Inc., Nutley, N.J.), given by thrice-weekly self-administered subcutaneous injections in the anterior thigh for 10 weeks (Fig. 3). Patients then underwent follow-up at weekly intervals for the first month, at bimonthly intervals for the next 2 months, and every 3 months thereafter.

Minor focal recurrences were managed by ablation in the office, usually with application of 85% trichloracetic acid. Patients with treatment failure from arms 1 and 2 were commenced on a regimen of 1 MIU thrice weekly for 10 weeks and those from arm 3 were in-

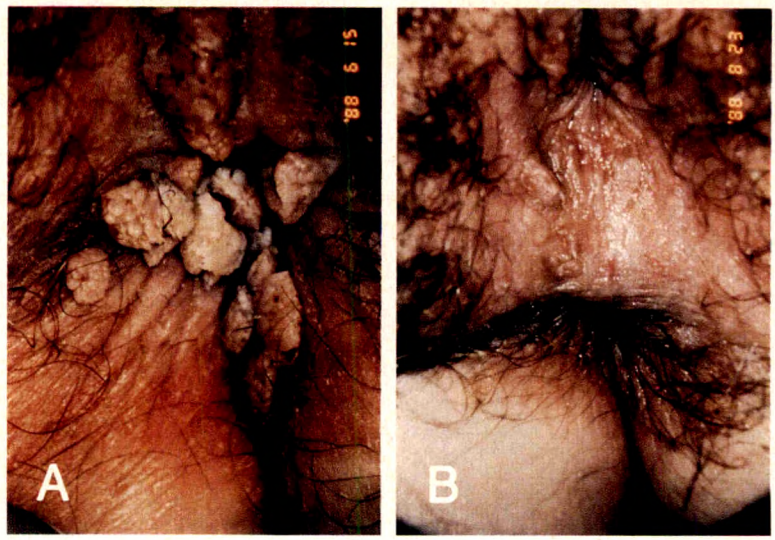


Fig. 4. A, Extensive condylomas have recurred by fourth postoperative week in patient initially treated by laser ablation alone. **B,** Complete response after crossover to 10 weeks of 1 MIU interferon plus adjuvant topical 5-fluorouracil.

Table I. Efficacy of laser ablation within each treatment arm, at 18-month anniversary of surgery

	Success		*Failure*		
Treatment arm	*No.*	*%*	*No.*	*%*	*Total*
Interferon	27	82	6	18	33
Topical 5-fluorouracil	9	50	9	50	18
Laser alone	8	40	12	60	20
TOTAL	44	62	27	38	71

$\chi^2 = 10.31$; $p < 0.002$ for interferon versus 5-fluorouracil–laser alone.

creased to 3 MIU interferon on the same dosage schedule. In this report results from the evaluable cases have been compiled into a contingency table reflecting status at the 18-month anniversary of surgery. Differences between regimens were analyzed by the χ^2 test.

Results

Eighty-four patients were originally scheduled with intent to treat: 26 in the untreated group, 29 in the 5-fluorouracil group, and 29 in the interferon group. However, between the time of constructing the randomization schedule and the time of actual surgery, two events arose to make it unethical to continue with the original intention to use 5-fluorouracil. First, analysis of historic data had already demonstrated that topical 5-fluorouracil was not an effective adjuvant, except in the presence of multiple risk factors.[4] Second, because of two case reports (Hoffmann-La Roche, Inc., data on file) of congenital malformations associated with intrauterine exposure to topical 5-fluorouracil, the Federal Drug Administration reclassified this drug as Category X (unsuitable for use in women of childbearing age). Consequently, 6 patients slated to receive adjuvant 5-fluorouracil received adjuvant interferon instead. Hence the final composition was 26 in the untreated group, 23 in the 5-fluorouracil group, and 35 in the interferon group.

After enrollment, 13 women were excluded: 3 for spontaneous regression before laser surgery and 10 due to protocol violations. This left 71 evaluable patients, 20 in the untreated arm, 18 in the 5-fluorouracil arm, and 33 in the interferon arm. Among the initially randomized patients, there were no significant differences with respect to mean age, age at first intercourse, number of sexual partners, duration of disease expression, number of anatomic sites involved, or number of adverse prognostic factors. At assessment of final outcome, it was found that 27 of 33 (82%) assessable patients in the adjuvant interferon arm were controlled by a single laser ablation, as compared with only 9 of 18 (50%) in the 5-fluorouracil group and 8 of 20 (40%) in the laser alone group (Table I). There was no statistical difference in outcome within the 5-fluorouracil and laser only arms. In contrast to 5-fluorouracil and laser alone, adjuvant interferon conferred a statistically significant protection against the frequency of postoperative recurrence (χ^2 10.31, $p < 0.002$). Moreover, we did not observe any side effects outside of the expected experience (i.e., some complaints of headaches and flulike symptoms).

Crossover of the 21 failures from arms 1 and 2 (to 1 MIU interferon and weekly 5-fluorouracil) secured complete regression in 18 (86%) instances (Fig. 4, Table II). Crossover to 3 MIU controlled 3 of the 6 failures

(50%) in arm 3. Although firm scientific inference cannot be drawn from these data, the need for further surgery in only 6 of these 27 failures (22%) stands in market contrast to our experience before the ready availability of interferon. Among the 160 patients treated between 1983 and 1987, 44 of 53 with initial failure (83%) required a second or third laser ablation.[4]

Comment

Large expanses of dermis can be safely removed with the carbon dioxide laser, with healing occurring by outgrowth from the underlying skin appendages rather than ingrowth from the wound edges.[13] Simultaneous removal of both the macroscopic papillomas and adjacent subclinical reservoir has produced the best results reported so far, controlling disease among 94% of the 160 most difficult referrals seen between 1982 and 1987.[4] However, the need for a second laser procedure in at least one third of the treated subjects speaks to both the strength and weakness of the carbon dioxide laser. The strength is that, used skillfully, the laser can remove any volume of diseased tissue under fine control, with an assurance of rapid healing and an absence of scarring. However, the weakness is that the laser cannot prevent reactivation of the latent viral reservoir within the adjacent skin during the postoperative healing phase. Hence the preliminary results from this open-label, randomized trial herald an important advance in the therapy of problematic human papillomavirus–associated vulvar disease. Used in conjunction with effective surgical debulking, adjuvant low-dose systemic interferon conferred a two-thirds reduction in postoperative recurrence during the first 6 months after extended laser ablation. In contrast, there was no protective effect attributable to the use of adjuvant 5-fluorouracil. It appears that the combination of skilled laser surgery plus adjuvant systemic interferon does produce results superior to those attainable by either laser alone or laser with adjuvant 5-fluorouracil.

Unlike the clear-cut efficacy of interferon when used in combination with surgery, it has been difficult to establish the value of this drug as a primary therapy. An effective topical regimen would provide local delivery without systemic toxicity. In spite of initial claims of a 90% complete response rate with natural leukocyte interferon ointment,[5] subsequent studies could not even confirm that topical preparations had biologic activity.[14] Intralesional administration does have a reproducible capacity to produce regression of injected warts while avoiding most systemic side effects.[15] However, success rates of intralesional regimens have been modest, perhaps no better than what could have been achieved by local destruction.[12] Moreover, the practicality of such regimens remains doubtful. Only a few papillomas can be injected during one course of treatment, and there is no effect on either uninjected lesions or the subclinical reservoir. In contrast, intramuscular and subcutaneous regimens have documented activity in extensive and refractory disease. In two small, open-label, noncomparative, dose-response studies, Gall et al.[7, 8] obtained complete response rates of up to 90% with doses of 5 MIU/m², albeit at a substantial cost in morbidity. All patients in the 5 MIU/m² category required dosage reduction because of the severity of side effects. Unfortunately, double-blind, placebo-controlled trials with better tolerated doses have been unable to replicate the complete resolution rates seen in earlier trials.[11] Although remarkable improvements can occur in selected patients, the predictability and durability of response as single agents have been disappointing. Moreover, about 20% of responders will experience relapse after discontinuing therapy. Although human papillomaviruses are the verified cause of a group of epithelial proliferations that sometimes progress to malignancy, the exact role that this relationship plays in the pathogenesis of invasive anogenital cancer is still uncertain.[16] Pending clarification of the high rate of latent infection among apparently healthy women, some have argued against the aggressive treatment of genital condylomas. However, we feel that the prevalence and distress occasioned by sexually transmitted human papillomavirus infection justify the treatment of this disease in its own right. Whereas stable clinical control is generally attainable with simple methods, there is a real need for an effective adjunct to laser ablation for difficult-to-manage cases. Whether 1 MIU represents the optimal choice of dosage requires further investigation.

Table II. Results of crossover among 27 with failure of initial treatment

Treatment group	*Initial failure*	*Crossover outcome*		
		Success		
		No.	*%*	*Failure*
Arms 1 and 2 (1 MIU interferon and 5-fluorouracil)	21	18	86	3
Arm 3 (3 MIU interferon and 5-fluorouracil)	6	3	50	3
TOTAL	27	21	78	6

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Therapeutic amniocentesis in twin-twin transfusion syndrome appearing in the second trimester of pregnancy

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Amniocentesis and drainage were performed in 19 twin pregnancies initially seen at <28 weeks' gestation with acute polyhydramnios caused by the twin-twin transfusion syndrome. The procedure was performed an average of three times for each patient (range one to six), with the removal of a mean volume of amniotic fluid of 2600 ml on each occasion (range 500 to 6700 ml). The interval between initial examination and delivery averaged 7 weeks (range 1 day to 18 weeks); 14 (37%) of the 38 babies survived the neonatal period. In the pregnancies with poor outcomes, the intertwin disparity in fetal size and the mean volume of amniotic fluid drained at each amniocentesis were greater than in the pregnancies in which one or both twins survived. (Am J Obstet Gynecol 1992;166:820-4.)

Key words: Twin-twin transfusion syndrome, amniocentesis, amniotic fluid drainage, polyhydramnios, ultrasonography

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In twin pregnancies with presumed twin-twin transfusion syndrome, development of acute polyhydramnios at <28 weeks' gestation is associated with a high perinatal mortality rate, primarily because of spontaneous abortion or very premature delivery of growth-retarded babies or babies with hydrops. In an analysis

Table I. Fetal survival in twin pregnancies initially seen with acute polyhydramnios at <28 weeks' gestation and managed conservatively or by amniocentesis

Management			
Conservative		*Amniocentesis*	
Author	*Survival rate*	*Author*	*Survival rate*
Chescheir and Seeds (1988)[1]	0/4	Bebbington and Wittmann (1989)[9]	0/6
Brown et al. (1989)[2]	1/8	Chescheir and Seeds (1988)[1]	0/6
Elejalde et al. (1983)[3]	0/2	Feingold et al. (1986)[10]	1/2
Patten et al. (1989)[4]	3/48	Brennan et al. (1982)[11]	1/2
Pretorius et al. (1988)[5]	1/14	Brown (1958)[12]	1/2
Urig et al. (1990)[6]	0/10	Brown (1980)[13]	2/2
Weir et al. (1979)[7]	0/16	Brown and Macaskill (1961)[14]	2/2
Wittmann et al. (1981)[8]	0/4	Danziger (1948)[15]	1/2
		Erskine (1944)[16]	1/2
		Gonsoulin et al. (1990)[17]	4/22
		Mills (1980)[18]	2/2
		Montan et al. (1985)[19]	2/4
		Nageotte et al. (1989)[20]	2/4
		Schneider et al. (1985)[21]	8/20
		Urig et al. (1990)[6]	6/18
TOTAL	5/106		33/96

Table II. Outcome of twin pregnancies initially examined with acute polyhydramnios and intrauterine death of one of the fetuses at <28 weeks' gestation

Gestation at presentation (wk)	*Dead fetus*	*Condition of surviving fetus*	*Outcome of pregnancy*	*wk*
20	Donor	Ventriculomegaly	TOP	20
19	Recipient	Hydrops	SA	20
21	Donor	Hydrops	LB	33
23	Donor	Normal	LB	32
27	Recipient	Normal	LB	37

TOP, Termination of pregnancy; *SA*, spontaneous abortion; *LB*, live birth.

of 51 such published cases in which no antenatal intervention was undertaken, only five babies survived (Table I). Several reports on a small number of patients have suggested that outcome may be improved by repeated amniocenteses; therefore during the last 3 years a systematic prospective study was undertaken to examine the possible efficacy of this approach.

Patients and methods

During a 3-year period (1988 to 1990) 27 twin pregnancies with polyhydramnios at <28 weeks' gestation were referred to our unit for prenatal diagnosis and management. Ultrasonographic examination demonstrated that the fetuses were discordant in size and were separated by a thin membrane; there was a single placenta. In each case the larger twin was surrounded by polyhydramnios and appeared to have a distended bladder. The smaller twin appeared to be fixed to the placenta because of oligohydramnios, and no bladder could be found.[22]

In five cases one of the twins was dead at initial examination, and the surviving cotwin had either ventriculomegaly ($n = 1$) or hydrops ($n = 2$) or was normal ($n = 2$). The outcome of these pregnancies is shown in Table II. In another case initially seen at 21 weeks the recipient twin was normal, but the donor had ventriculomegaly. The pregnancy was managed conservatively and resulted in a spontaneous abortion at 22 weeks. Postmortem examination demonstrated intraventricular hemorrhage in the donor twin.

In the remaining 21 patients both fetuses were normal. The parents were counseled as to the likely poor prognosis and offered the options of conservative management, decompression amniocentesis, or termination of pregnancy. One patient opted for conservative management and miscarried 1 week after examination; another requested termination of pregnancy. The other 19 patients consented to serial amniocenteses. Ultrasonographically guided amniocentesis was performed with an 18-gauge needle, and amniotic fluid was al-

Table III. Gestational age at amniocentesis, volume of amniotic fluid, drained, pregnancy outcome, intertwin difference in abdominal circumference at initial examination

	Volume drained (L)										
Case No.	*At ≤20 wk*	*At 21 wk*	*At 22 wk*	*At 23 wk*	*At 24 wk*	*At 25 wk*	*At 26 wk*	*At 27 wk*	*At 28 wk*	*At 29 wk*	*At ≥30 wk*
1	—	—	—	6.7	—	—	—	—	—	—	—
2	—	—	—	2.5	—	—	—	—	—	—	—
3	—	—	—	3.0	2.4	—	—	—	—	—	—
4	—	—	—	3.2	4.0	—	—	—	—	—	—
5	1.0	—	—	—	—	—	—	—	—	—	—
6	—	—	—	2.5	2.3	—	3.0	—	—	—	—
7	—	—	3.5	—	—	0.5	—	—	—	—	—
8	—	4.0	2.0	1.7	2.0	—	—	—	3.0	—	—
9	—	—	5.0	—	—	—	—	4.9	4.6	6.0	—
10	—	—	4.0	—	3.5	—	1.5	3.5	—	3.0	—
11	—	—	3.0	2.8	1.0	2.5	—	—	—	—	—
12	—	—	—	—	1.5	—	—	3.7	—	—	—
13	—	—	2.1	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	3.0	3.0	1.5	4.3	3.2
15	—	3.2	—	1.5	—	3.2	—	1.9	—	—	—
16	—	4.5	—	—	2.4	3.3	—	2.8	—	—	—
17	0.6	0.5	—	—	—	—	—	—	—	—	—
18	1.0	3.0	—	3.0	—	—	—	3.0	—	3.0	—
19	1.0	1.5	1.0	1.0	1.0	1.5	—	—	—	—	—

SA, Spontaneous abortion; *IUD*, intrauterine death; *NND*, neonatal death; *LB*, live-born survivor.
*Died at 6 months.

lowed to drain freely into a sterile bag through a plastic tube attached to the hub of the needle. In each case 500 to 6700 ml was drained over a period of 40 to 120 minutes until there was subjective normalization of amniotic fluid volume on ultrasonographic examination. Patients were assessed every week; if they had symptoms or if the abdomen was considered to be tense on palpation, further drainage was performed.

Results

For the 19 patients who had amniocentesis, the gestation at examination, volume of amniotic fluid drained, and outcome are summarized in Table III. The mean gestation at initial examination was 21 weeks 5 days (range 17 to 25). Amniocenteses were performed one to six times (mean three) with the average removal of 2600 ml of amniotic fluid at each tap (range 500 to 6700 ml). The mean interval between initial examination and delivery was 6.5 weeks (range 1 day to 18 weeks).

Four patients (Table III; cases 1 through 4) aborted spontaneously within 48 hours of the first or second drainage procedures. In six patients (Table III; cases 5 through 10), amniocentesis and relief of polyhydramnios were assoicated with prolongation of pregnancy by 2 to 9 weeks, but either both babies died in utero or the death of one fetus was associated with deterioration in the condition of the second twin, who was delivered but died in the neonatal period. In four cases (Table III; cases 11 to 14) one infant survived, but the other twin died either in utero or in the neonatal period. In five cases (Table III; cases 15 to 19) both babies survived the neonatal period; one infant died at the age of 6 months.

The mean gestational age at initial examination in the nine pregnancies in which at least one child survived (21^{+3} weeks) was similar to that of the 10 pregnancies in which both twins died (21^{+6} weeks). However, unsuccessful pregnancies were associated with a greater intertwin disparity in abdominal circumference at initial examination (Fig. 1; 33% compared with 15% in successful pregnancies). Furthermore, in pregnancies with poor outcomes the mean volume of amniotic fluid removed at each procedure was higher (3200 ml compared with 2300 ml in successful pregnancies).

Comment

In twin pregnancies with acute second-trimester polyhydramnios treated by amniocentesis the overall fetal survival rate of 37% compares favorably with the results of series in which there was no antenatal intervention (Table I). The most likely mechanism by which decompression amniocentesis may improve survival is by prolongation of pregnancy through prevention of the polyhydramnios-mediated risk of spontaneous abortion or very premature delivery. It is also possible that with advancing gestation and increasing fetoplacental blood volume the relative significance of the hemodynamic effects of placental anastomoses is reduced. In this respect repeated amniocenteses may, at least in

Donor		Recipient		Difference in abdominal circumference (%)
Outcome	wk	Outcome	wk	
SA	23	SA	23	30
SA	23	SA	23	30
SA	24	SA	24	50
SA	24	SA	24	23
IUD	19	IUD	19	21
IUD	28	IUD	29	74
NND	26	NND	26	23
NND	28	IUD	28	22
IUD	29	NND	29	43
NND	31	IUD	30	14
LB	31	IUD	26	9
LB	30	IUD	26	8
LB	27	NND	27	11
NND	30	LB	30	2
LB	28*	LB	28	40
LB	29	LB	29	7
LB	35	LB	35	20
LB	36	LB	36	26
LB	37	LB	37	10

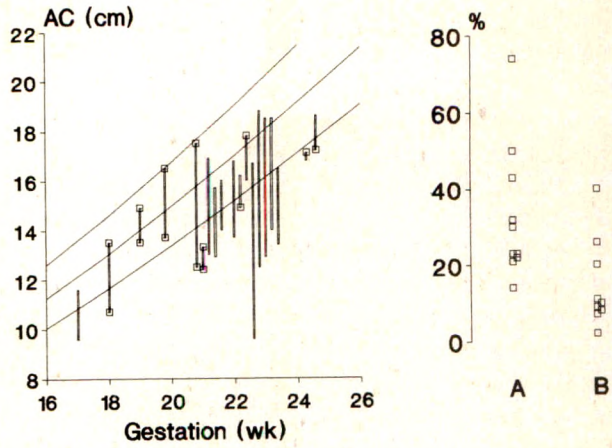


Fig. 1. Fetal abdominal circumferences *(AC)* in 19 twin pregnancies with acute second-trimester polyhydramnios treated with decompression amniocenteses plotted on reference range for gestation *(left)*. Of 38 fetuses, 24 died in utero or in neonatal period and 14 survived *(left, boxes)*. Intertwin difference in abdominal circumference at initial examination (expressed as percentage of smaller twin) was greater in pregnancies resulting in deaths of both babies *(A)* than in pregnancies in which one or both babies survived *(B; right)*.

some cases, allow the fetal cardiovascular system to outgrow the deleterious effect of placental anastomosis.[22]

These data challenge the assertion that in twin-twin transfusion syndrome the need for decompression is a poor prognostic feature and that treatment by amniocentesis adds nothing to the management of this condition.[1, 9] However, the apparent beneficial effect of decompression amniocentesis may merely reflect differences in reported study populations. Thus retrospective studies on the conservative management of twin-twin transfusion syndrome often use the criterion of a major disparity in fetal weight to identify the population from hospital records. In contrast, prospective intervention studies tend to select patients purely on the basis of polyhydramnios, and the issue of disparity in fetal weight is generally dealt with in a less stringent manner. Our data indicate that the greater the disparity in size between twins at initial examination the worse the prognosis. The reported outcome of conservative management is poorer than that of interventional management (Table I); the difference may be partly explained by the inclusion of pregnancies with larger intertwin differences in the conservatively managed group. This, coupled with the phenomenon of reporting bias (a greater likelihood to publish cases in which intervention appeared to be successful) may have exaggerated the apparent advantages of treatment.

The association between poor outcome and large intertwin differences in size and the need for drainage of larger volumes of amniotic fluid is presumably a consequence of the severity of the underlying disease. Twin-twin transfusion syndrome is traditionally believed to be caused by the unbalanced shunting of blood from donor to recipient, resulting in anemia and polycythemia, respectively. However, fetal blood sampling by cordocentesis has recently demonstrated that there are no major intertwin differences in hemoglobin concentration, and an alternative hypothesis has been suggested regarding the pathogenesis of this condition.[23] Both the severe early onset growth retardation in the donor and the polyhydramnios in the recipient twin may be consequences of uteroplacental insufficiency affecting the donor twin. Increased peripheral resistance in the placental circulation of the donor twin would promote the shunting of blood to the recipient twin through the vascular anastomoses that are invariably present in all monochorionic twin pregnancies.[24] The recipient twin is able to dispose of the additional fluid load by increased urine production,[22] but the protein and cellular components remain in its circulation to create an increase in colloid osmotic pressure that causes water to move from the maternal compartment across the placenta. Thus volume overload and diuresis continue, and a vicious cycle is established. The more severe the uteroplacental insufficiency, the earlier the appearance of symptoms, the smaller the donor twin, the larger the intertwin disparity in size, the worse the polyhydramnios, and the poorer the prognosis.

Increased intrauterine pressure as a consequence of marked polyhydramnios would be expected to compromise the placental circulation further. Since we analyzed our results, Elliott et al.[25] have reported a perinatal survival rate of 79% in twin-twin transfusion syndrome treated with repeated amniocenteses; they

attribute the high survival rate to improved placental blood flow after decompression.[25] The lower rate of fetal salvage in our series may be caused by differences in the two study populations because the treatment protocols appear to have been similar.

Selective feticide has been suggested as an alternative form of therapy in twin-twin transfusion syndrome.[26] Spontaneous intrauterine death of one twin may to some extent represent a parallel situation with regard to the likely outcome in the surviving fetus. In three of the five cases in our series in which one fetus was dead at the time of initial examination at 21 to 27 weeks' gestation, there was gradual resolution in polyhydramnios and the healthy cotwins were born 9 to 12 weeks later; in one case hydropic changes resolved in the weeks after initial examination (Table II). However, in another two cases the surviving twin developed ventriculomegaly. Although cerebral damage in surviving twins has been attributed to disseminated intravascular coagulation after the release of thromboplastin from the dead twin, this may not be the underlying mechanism in all cases; we have also observed ventriculomegaly caused by intraventricular hemorrhage in one donor fetus while both twins were alive. In five of the pregnancies treated with amniocentesis one of the fetuses died; in none of these cases was there an obvious improvement in either the polyhydramnios or the condition of the surviving twin. Indeed, in three cases the condition of the other twin deteriorated, and the baby died either in utero or in the neonatal period.

Laser occlusion of placental anastomosis[27] may convincingly be shown to improve the outcome of twin-twin transfusion syndrome appearing in the second trimester of pregnancy. In the meantime, we recommend decompression amniocentesis as an easy and relatively safe option providing some hope in an otherwise hopeless situation.[10] Nevertheless, the value of such treatment can only be established if factors of prognostic significance are clearly defined. Intertwin disparity in abdominal circumference at initial examination appears to be one such factor and should be taken into account when considering the apparent efficacy of therapy.

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Immunohistochemical detection of the multi-drug-resistance marker P-glycoprotein in uterine cervical carcinomas and normal cervical tissue

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Uterine cervical carcinomas and normal cervical tissue (controls) were investigated for the presence of the multi-drug-resistance gene product P-glycoprotein by means of immunohistochemistry, with the C219 monoclonal antibody and the streptavidin-biotin-peroxidase technique. Ten of 11 cervical carcinomas, 2 of which were previously treated with chemotherapeutic agents of the multi-drug-resistance group, showed a positive reaction of tumor cells. All normal controls showed a positive reaction of the ectocervical and endocervical epithelial cells. P-Glycoprotein seems to be implicated at least in part in resistance to chemotherapy of cervical carcinoma. (Am J Obstet Gynecol 1992;166:825-9.)

Key words: P-Glycoprotein, carcinoma, uterus, cervix

The multi-drug-resistance phenotype is characterized by tumors exhibiting cross-resistance toward a variety of pharmacologically unrelated chemotherapeutic compounds. Among these are the anthracyclines (doxorubicin, its derivatives, and daunorubicin), the vinca alkaloids (vincristine and vinblastine), the podophyllotoxins (etoposide and teniposide), actinomycin D, and colchicine, most of which are widely used in clinical practice.[1-3] Multidrug resistance can be elicited in experimental tumors in vitro by exposing them to just one of the agents cited above; tumor cells frequently become cross-resistant toward the complete set of these drugs.[4] Multidrug resistance is associated with the overexpression of a membrane pump, called P-glycoprotein or P-170, because of its molecular weight of approximately 170 kd, which actively extrudes chemotherapy in a nonspecific fashion from the interior of the cell.[5] The involvement of P-glycoprotein in the development of the multi-drug-resistance phenotype has been convincingly demonstrated by the conversion of sensitive cells into multi-drug-resistant ones by transfection of the gene encoding P-170.[6] In humans it is known that tumors arising on organs with high intrinsic levels of P-glycoprotein, such as the kidney, colon, or liver, are also highly chemoresistant, not only toward chemotherapy belonging to the multi-drug-resistance family but to virtually any form of chemotherapy.[1-3]

An association between increased P-glycoprotein expression and development of resistance to therapy, analogous to the one extensively studied in experimental tumors, has been demonstrated in clinical specimens. These include gynecologic tumors, such as those found in the ovary,[7, 8] We have recently reported that endometrial carcinoma, which is somewhat refractory to conventional chemotherapeutic treatment, expresses P-glycoprotein at demonstrable levels before treatment.[9] Chemotherapy is being used increasingly in cervical carcinoma, not only for treatment of advanced disease but also as neoadjuvant therapy in operable cases.[10, 11] Many of the agents used for this purpose belong to the multi-drug-resistance class. Thus it would seem pertinent to analyze cervical carcinoma for the expression of P-glycoprotein. Therefore we studied a series of treated and untreated cervical carcinoma specimens, together with normal cervical tissue as a control, with immunohistochemistry, a method we have found useful previously in mammary, endometrial, and ovarian carcinomas.[9, 12]

Material and methods

Eleven cervical carcinomas and 10 normal controls were analyzed for the expression of P-glycoprotein. The specimens were obtained from patients of the Department of Obstetrics and Gynecology of the University Clinic (Hospital de Cruces) of Bilbao, Spain, either at biopsy or at the time of operation. Two patients received two and three courses, respectively, of polychemotherapy containing vincristine, a member of the multi-drug-resistance family, before the tumor material

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Table I. Cervical carcinoma: P-Glycoprotein reactivity with monoclonal antibody C219

Patient No.	*Age (yr)*	*Histologic type*	*Stage*	*Treatment*	*P-Glycoprotein*
1	47	Squamous	IB	None	—
2	42	Adenosquamous	IB	Vincristine-bleomycin-cisplatin (3 courses)	+ +*
3	43	Squamous	IV	Vincristine-bleomycin-cisplatin (3 courses)	+ +†
4	52	Squamous	IB	None	+†
5	46	Squamous	IB	None	+*
6	32	Squamous	IB	None	+†
7	45	Squamous	IIA	None	+ +†
8	58	Squamous	IB	None	+†
9	32	Adenocarcinoma	IIA	None	+†
10	50	Adenocarcinoma	IIA	None	+ +*
11	36	Minimal deviation adenocarcinoma ("adenoma malignum")	IIIA	None	+ + +†

−, No reaction; +, weak reaction; + +, strong reaction, comparable to that of positive controls; + + +, very strong reaction, of higher intensity than that of positive controls.

*5% to 20% Positive tumor cells.

†>20% Positive tumor cells.

Table II. Normal cervical controls: Reactivity with monoclonal antibody C219

Specimen No.	*P-Glycoprotein reactivity*	
	Ectocervix	*Endocervix*
1	+	+ +
2	+ +	+ +
3	+ +	+ +
4	+	+ +
5	+	+
6	+ +	+ +
7	+ +	+ +
8	+	+
9	+ +	+ +
10	+ + +	+ + +

+, Reaction of lower intensity than that of the positive controls; + +, reaction equal to that of positive controls; + + +, reaction stronger than that of positive controls.

was obtained. The clinical characteristics of the patients and histologic data of the tumors are summarized in Table I.

Immunohistochemistry (streptavidin-biotin-peroxidase method) was performed with the C219 monoclonal antibody originally developed by Kartner et al.,[13] which recognizes an epitope of P-glycoprotein located on the inner side of the cell membrane. The technical details have been described extensively elsewhere.[9, 12] Briefly, the specimens were immediately snap-frozen in liquid nitrogen at the time of surgery or biopsy and stored at −70° C until processed. Cryostat sections, 6 μm thick, were then made and fixed in cold acetone at −20° C for 10 minutes. The sections were rehydrated in phosphate-buffered saline solution, then incubated with normal serum from the same species that the second, bridge antibody is derived from, for the blocking of nonspecific binding. The monoclonal first antibody (C219, Centocor, Malvern, Pa.) was applied at concentrations varying from 2.5 to 10 μg/ml and incubated overnight at 4° C. The best results were obtained at a concentration of 10 μg/ml. The second biotinylated antimouse antibody (1:50) and the streptavidin-biotinylated-peroxidase complex (1:100) were applied in successive steps. Staining was done with 3-amino-9-ethylcarbazole, giving a red reaction product. The preparations were finally counterstained with Mayer's hematoxylin and mounted with glycerol gelatin. Negative controls for each sample were carried in parallel by incubation with normal mouse serum in substitution for the first antibody at the same concentration, and the rest of the procedure was carried out as described. As positive controls we used doxorubicin-resistant sarcoma-180 cell smears and preparations from a solid sarcoma-180 xenograft resistant to daunorubicin, expressing high levels of P-glycoprotein.[4]

Results

Of the 11 cervical carcinomas studied, 10 showed a clearly positive P-glycoprotein reactivity (Table I). The intensity of the reaction varied among four different grades: no reaction (−), one squamous carcinoma; discernible but lower than the one found in the positive controls (+), four squamous carcinomas and one adenocarcinoma; high, comparable to that of the positive controls (+ +), two squamous carcinomas (Fig. 1, *A*), one adenosquamous carcinoma (Fig. 1, *C*), and one adenocarcinoma; extremely high, i.e., stronger than the positive controls (+ + +), one case of minimal deviation adenocarcinoma, or "adenoma malignum" (Fig. 2, *A*). Both cases previously treated with polychemotherapy containing vincristine, a drug of the multi-drug-resis-

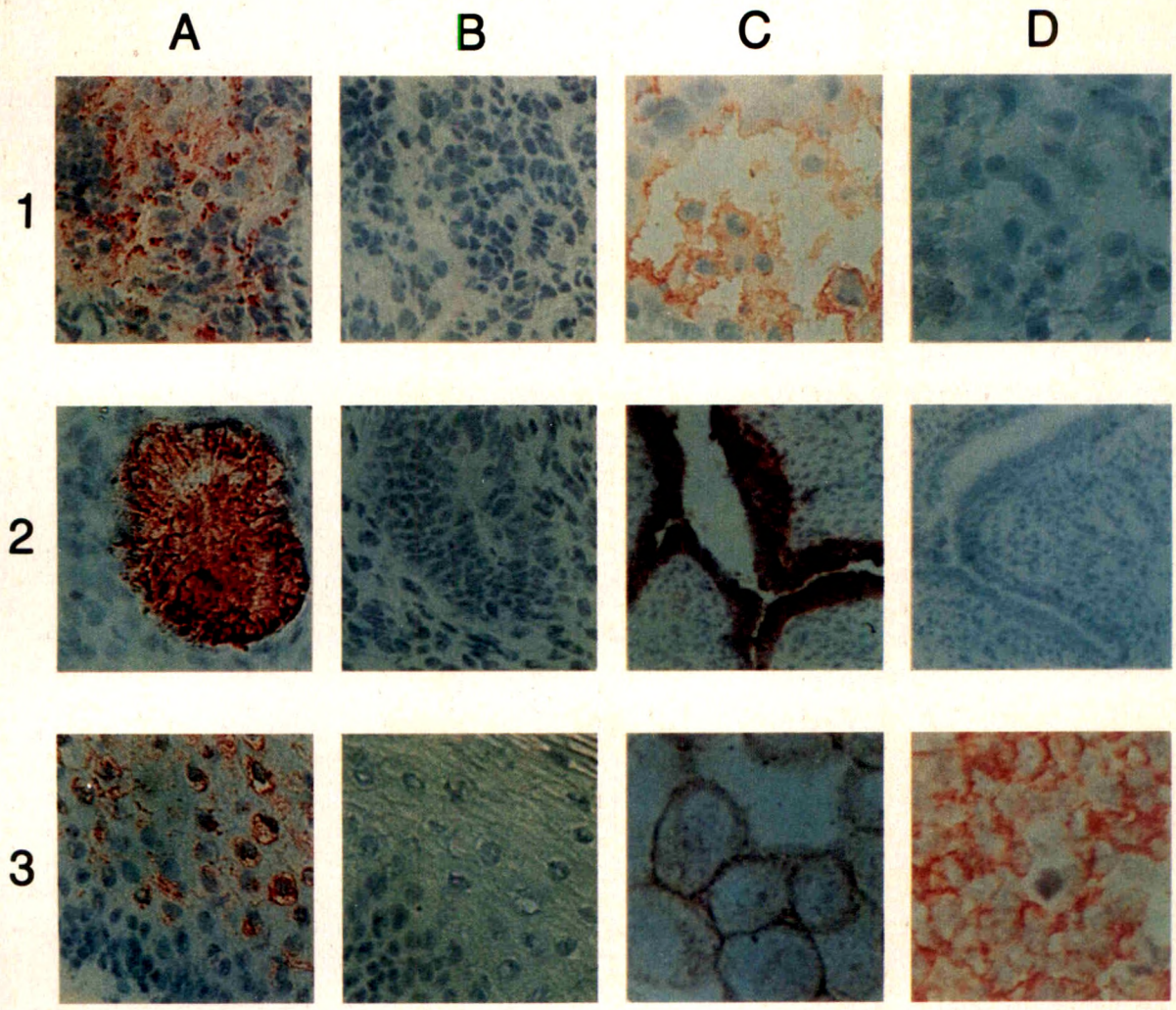


Fig. 1. P-glycoprotein reactivity with monoclonal antibody C219, streptavidin-biotin-peroxidase method. **A,** Squamous carcinoma of uterine cervix. **B,** Negative control of 1, *A*. **C,** Adenosquamous carcinoma of cervix (adenomatous portion). **D,** Negative control of 1, *C*.

Fig. 2. P-glycoprotein reactivity with monoclonal antibody C219, streptavidin-biotin-peroxidase method. **A,** Minimal deviation adenocarcinoma (adenoma malignum) of cervix. **B,** Negative control of 2, *A*. **C,** Normal endocervical glandular epithelium. **D,** Negative control of 2, *C*.

Fig. 3. P-glycoprotein reactivity with monoclonal antibody C219, streptavidin-biotin-peroxidase method. **A,** Normal ectocervical squamous epithelium. **B,** Negative control of 3, *A*. **C,** Multi-drug-resistant sarcoma-180 cells. **D,** Nude rat solid xenograft of multi-drug-resistant sarcoma-180 cells.

tance family, belonged to the second group, with a high reactivity (++). As to the distribution of positive cells (Table I), three of the 10 positive cases had only between 5% and 20% positive cells, whereas the remaining seven cases showed reactivity in >20% of the tumor cells. Of these, in the case of minimal deviation adenocarcinoma (tumor No. 11) with an immunohistochemical reaction of higher intensity than the controls (+++), virtually 100% of the tumor cells were positive (Fig. 2, *A*).

Both the ectocervical squamous and the endocervical glandular epithelium of the normal cervical specimens from the control group (Figs. 2, *C*, and 3, *A*) were reactive with the C219 monoclonal antibody (Table II). The reaction showed the same variations in intensity as described for the tumors. The distribution, however, was more uniform than the one that was observed in tumors, all endocervical glands (Fig. 2, *C*) and >20% of ectocervical cells (Fig. 3, *A*) being positive in all cases.

Comment

The results of this series seem to indicate that P-glycoprotein is expressed in a high number of cervical carcinomas. We found it present in 10 of 11 cases studied, regardless of the histologic type. P-Glycoprotein was also detected on the epithelium of all normal cervical controls, both on the endocervical glands, which

expressed it at very high levels, and on the squamous epithelium of the ectocervix. Riou et al.,[14] by means of molecular biology, recently investigated the content of messenger ribonucleic acid (mRNA) encoding P-glycoprotein in cervical carcinoma and normal cervical epithelia. They found elevated levels in 88% of treated carcinomas, 69% of adenocarcinomas, and 39% of untreated squamous carcinomas. With the possible exception of untreated squamous carcinomas, all these findings were comparable to ours. The fact that we found a higher number of positive cases overall may be attributed to several reasons. Our series is relatively small, and, however consistent the results seem to be, it is likely that a larger number of cases studied would also yield a higher percentage of negative ones. On the other hand, the method used by Riou et al.,[14] i.e., slot blot analysis of mRNA content, in spite of a very high sensitivity, has an inherent limitation that the authors have pointed out: The material analyzed by slot blotting is a homogenate of tissue, containing not only tumor cells but also a variable stromal and normal tissue component. If the latter is large enough, it may well dilute the content of target mRNA from the tumor (or colonies of positive cells within it) and thus produce a false-negative result. Three of our 10 positive tumors had only between 5% and 20% cells that were reactive for P-glycoprotein, and the reaction was weak in two of the three cases (Table I). It is likely that some or all of these samples would have produced a negative result if analyzed for mRNA, especially considering that all our specimens contained significant (and often predominant) portions of stroma or normal tissue. Both myometrium and isthmus in our normal control biopsy specimens, for instance, have been consistently negative for P-glycoprotein in this series, although representing the largest portion of tissue present on each preparation. All this may well explain the only significant discrepancy between our results and those reported by Riou et al.,[14] who found only 39% positive cases among their untreated squamous cervical carcinomas, whereas five of our six cases (83%) were positive. However, of our five positive cases, only one (20%) showed a strong (++) reaction, whereas the other four, with a weak reaction in the tumor cells and a significant proportion of nonreactive stromal and normal cervical tissue, could easily yield a false-negative result if analyzed for mRNA. In this context we have referred in previous articles[9, 12] to the relative advantages of immunohistochemistry for the study of P-glycoprotein expression in clinical tumor specimens. In addition to being cheaper and less complicated to perform than the sophisticated methods of molecular biology, it has the added advantage of preserving the histologic structure of the tissues studied. It thus allows researchers to discern which cells do express P-glycoprotein and whether these are tumor cells. On the other hand, it has the drawbacks of lower sensitivity and of being a purely qualitative method, not allowing quantitation of the results obtained.

On the basis of the current study it may be concluded that cervical carcinoma belongs to the category of tumors arising from organs with high intrinsic levels of P-glycoprotein, and these tumors also tend to be highly chemoresistant. It has been postulated that all these organs, which have in common excretory and detoxifying functions, overexpress P-glycoprotein as part of a wide array of defense mechanisms and that chemoresistance is only a byproduct of them.[1-3] Cervical adenocarcinoma, which originates on a glandular epithelium, would fit well into this model. Why the squamous epithelium of the cervix, which has no known detoxifying or excretory function, also expresses P-glycoprotein, giving rise to tumors with the multi-drug-resistance phenotype, is a mystery it shares with connective tissue, which, being equally nonspecific in function, gives rise to sarcomas that express high levels of P-glycoprotein.[15] It is, however, coincident with the clinical finding that cervical carcinoma tends to be minimally responsive to chemotherapy and that the few agents to which it responds best do not belong to the multi-drug-resistance type.[10] Indeed, in a prospective study conducted by the Gynecologic Oncology Group, it was concluded that the addition of vincristine (a multi-drug-resistance type), mitomycin C, and bleomycin to cisplatin in the treatment of advanced or recurrent cervical carcinoma was not superior to cisplatin alone.[16] In the light of this report, the astonishingly high response rates reported by Giaroli et al.[11] for neoadjuvant therapy with three preoperative courses of vincristine, bleomycin, and cisplatin may be attributed mainly to cisplatin and to a lesser degree to bleomycin and the multi-drug-resistance agent vincristine. Moreover, the single drug eliciting the highest response rate in all published series is cisplatin,[17] and it is well known from experimental studies that multi-drug-resistant tumor cells tend to retain their sensitivity to cisplatin because the resistance to this agent is probably mediated by other mechanisms, such as glutathione–*S*-transferase,[18] which are completely different from P-glycoprotein.

There are some clinical implications to be derived from our findings. In view of them, it would seem of little value to use chemotherapy of the multi-drug-resistance class for the treatment of cervical carcinoma. This is substantiated by previous clinical trials, as revised by Thigpen et al.,[17] where these agents elicited very low response rates when used singly. In addition, if we may extrapolate from the experience with other tumors derived from organs with high intrinsic levels of P-glycoprotein (kidney, liver, colon, etc.), even the use of agents not related to the multi-drug-resistance

phenomenon will probably never attain the effectiveness reached in chemosensitive tumors, such as mammary and ovarian carcinoma, which, of significance, express P-glycoprotein in a much lower proportion of cases before treatment.[9, 12, 19] A theoretic way out of this dilemma may be the circumvention of multidrug resistance with drugs inhibiting P-glycoprotein function. A number of membrane-active compounds, such as verapamil and trifluoperazine, are able to completely reverse multidrug resistance mediated by P-glycoprotein when used under experimental conditions.[20] The doses needed for this, however, far exceed the therapeutic dose tolerated in humans; this represents the major obstacle to their clinical use.

In conclusion, P-glycoprotein was demonstrated with immunohistochemistry techniques in most cervical carcinomas studied. It was also present on the epithelium of all normal cervical controls. P-Glycoprotein may thus play a significant role in the known resistance to chemotherapy of cervical carcinomas, although other mechanisms of resistance must be implicated.

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A randomized trial of prostaglandin E_2 in a controlled-release vaginal pessary for cervical ripening at term

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OBJECTIVE: The purpose of this study was to determine if prostaglandin E_2 in a controlled-release vaginal pessary can produce cervical ripening at term.
STUDY DESIGN: This was a double-blind, randomized, placebo-controlled study conducted at a university center and involving 81 patients with 42 receiving active agent. Categoric data were analyzed by Pearson's χ^2 or logistic regression. Continuous variables were analyzed by analysis of variance and the F test.
RESULTS: Prostaglandin E_2 was significantly better than placebo at cervical ripening and at decreasing the time to rupture of membranes, the time to onset of labor, the need to give oxytocin, and the time to vaginal delivery. Multiparous women benefitted more than primiparous ones. The cesarean section rate decreased only for multiparous women. Uterine hyperstimulation occurred only with prostaglandin E_2 and after the onset of labor.
CONCLUSIONS: Prostaglandin E_2, when administered in a controlled-release vaginal pessary, is affective in producing cervical ripening at term. This agent should be used on inpatients who are under continuous monitoring and it should be removed at the onset of labor. (Am J Obstet Gynecol 1992;166:830-4.)

Key words: Pregnancy, cervical ripening, induction of labor, PGE_2, prostaglandin

Prostaglandin E_2 (PGE_2) has been suggested as an agent to ripen the unfavorable cervix for induction of labor at term. It has several properties that might make it a likely candidate for this task. It can relax the smooth muscle of the cervix[1] and induce an increase in the production of glycosaminoglycans by cervical fibroblasts.[2] The increased glycosaminoglycans may be involved in cervical softening unrelated to uterine contractions, and such softening and increased extendibility have been associated with exogenous PGE_2 application.[3]

Unfortunately, for its role as a cervical ripening agent, PGE_2 is also a uterotonic agent for the pregnant uterus and there are concerns about production of hyperstimulation with its use.[2] PGE_2, when applied in a gel intracervically, has been documented to produce cervical ripening and occasional hyperstimulation.[4] Because PGE_2 is rapidly metabolized in the tissues of origin and by the lungs and gastrointestinal tract, if it were possible to remove the exogenous PGE_2, hyperstimulation could be rapidly reversed. A readily removable solid intravaginal dosage form would be welcome. This report describes a blinded, placebo-controlled trial of a new controlled-release intravaginal PGE_2 pessary for preinduction cervical ripening at term.

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Material and methods

This study was approved by our institutional review board for human trials and consisted of a randomized, blinded, placebo-controlled trial of an intravaginal PGE_2 pessary for cervical ripening. Change in cervical status before induction was assessed by the Bishop score, as described in Bishop's classic article.[5]

The pessary is made of a cross-linked polymer hydrogel that releases PGE_2 at a near-constant rate of approximately 1 mg/hr in vitro. The total reservoir contained in the pessary was 10 mg. It was supplied together with placebo pessaries in foil wrappers, which were kept at −20° C until immediately before use.

The patients were recruited from women to be delivered at our hospital who were to have labor induced at term and who fulfilled the eligibility criteria listed in Table I.

After giving informed consent, the patient underwent a 30-minute observation period in which a nonstress test was performed. If this test was reactive and the patient met all other criteria, she entered the study and was randomly assigned to receive either active or placebo pessary. At the time of pessary insertion, a Bishop score was obtained and the thawed pessary was inserted into the posterior fornix of the vagina with its long axis transverse to the long axis of the vagina. The patient was asked to remain recumbent for the first 2

Table I. Eligibility criteria

1. Informed consent
2. Singleton pregnancy of ≥37 weeks' gestation
3. Cephalic presentation
4. Nulliparous or multiparous with ≤3 previous viable infants
5. A medical or obstetric indication for induction
6. Bishop score of ≤4 on admission
7. Reactive fetal nonstress test
8. No previous uterine scars
9. No vaginal bleeding
10. Intact membranes or premature rupture of membranes for ≤4 hours at insertion of pessary
11. No fever
12. No allergy to prostaglandins
13. No history of asthma or glaucoma, except for childhood asthma without adult recurrences
14. No evidence of fetal distress
15. No evidence of spontaneous labor
16. No evidence of clinical hydramnios, confirmed by ultrasonography
17. No use of aspirin, nonsteroidal antiinflammatory drugs, or acetaminophen within 4 hours before insertion of pessary and any time thereafter
18. No underlying maternal cardiac lesion that might put patient at risk from potential hypotensive effects of PGE_2

Table II. Demographic variables of patients enrolled

	Placebo	*Active agent*	*p Value**
Race			
Black	21	29	0.3230
White	16	13	
Native American	1		
Oriental	1		
Age (yr)			
Mean ± SD	23.1 ± 5.79	23.7 ± 5.35	0.6496
Range	15-40	14-33	
Height (m)			
Mean ± SD	1.6 ± 0.07	1.6 ± 0.05	0.2245
Range	1.5-1.8	1.5-1.8	
Weight (kg)			
Mean ± SD	86.1 ± 17.13	84.6 ± 17.61	0.7048
Range	55-132	48-125	

**p* Values for categoric data from Pearson's χ^2 test. *p* Values for continuous data from one-way analysis of variance F test.

hours after insertion and then was allowed to get up as needed. The pessary was to remain in situ for 12 hours, during which continuous electronic monitoring was used to observe uterine activity and fetal heart rate. Repeat Bishop scores were performed by the same individual if possible at 6 and 12 hours after insertion. If, for any reason, the pessary had to be removed before 12 hours after insertion, a repeat Bishop score was obtained at removal. All pessaries still in situ at the time of the 12-hour Bishop score were removed at that time. After removal of the pessary, any patient who was not in labor underwent induction of labor with oxytocin. Amniotomy was performed at the discretion of the attending physician.

Eighty-one patients entered the study. Thirty-nine received placebo and 42 received the active agent. Of the original 81 patients, 36 placebo and 41 active-agent patients could be evaluated for cervical ripening. To be evaluated for cervical ripening the patient must have been eligible for the study and have had at least one Bishop score after the initial one. Of the placebo patients who could not be evaluated, one never received a pessary when it was determined after randomization that she had a uterine scar and was ineligible, the second patient had insufficient data to analyze because she had a cesarean delivery at 7½ hours after insertion for worsening severe preeclampsia, and the third had ruptured membranes for >4 hours at pessary insertion and was ineligible. The active-agent patient who could not be evaluated was ineligible because she had onset of labor at pessary insertion.

In addition to cervical ripening, the labor parameters that were of interest included time to rupture of membranes, time to onset of labor, length of the first stage of labor, length of the second stage of labor, and the time to vaginal delivery. Labor was defined as uterine contractions occurring at least every 3 minutes and lasting at least 45 seconds or cervical dilatation of 5 cm with any contraction pattern. All times were measured from pessary insertion, with the exception of the first and second stages of labor. Thirty placebo patients and 39 active-agent patients could be evaluated for these

Table III. Obsteric history of patients enrolled

	Placebo	*Active agent*	*p Value**
Gestational age (wk)			
Mean ± SD	39.9 ± 1.69	39.6 ± 1.38	0.3034
Range	37-43	37-42	
Prior pregnancies			
0	32	26	0.0810
1	4	12	
2	3	2	
3		2	
Initial Bishop score			
Mean ± SD	2.9 ± 1.08	2.8 ± 1.03	0.8704
Range	0.0-4.0	1.0-4.0	

**p* Values for categoric data from Fearson's χ^2 test. *p* Values for continuous data from one-way analysis of variance F test.

Table IV. Primary indication for induction*

	Placebo	*Active agent*
Decreased amniotic fluid	5	5
Decreased fetal movement	1	0
Diabetes mellitus	4	2
Elective	2	1
Hypertension	7	11
Impending macrosomia	1	3
Intrauterine growth retardation	3	5
Postterm	11	11
Premature rupture of membranes	1	2
Sickle cell disease	1	0
Preeclampsia	3	2

**p* = 0.8300 by Pearson's χ^2 test.

Table V. Measures of successful cervical ripening*

	Placebo	*Active agent*	*p Value†*
Treatment outcome			
Success	10	30	0.0012
No success	26	11	
Change in Bishop score ≥3			
Yes	8	23	0.0041
No	28	18	
Bishop score ≥6			
Yes	10	25	0.0062
No	26	16	
Vaginal delivery within 12 hr			
Yes	0	12	0.0615
No	36	29	
Onset of labor within 12 hr			
Yes	0	24	0.0005
No	29	11	
Not available	7	6	

*Patients may meet more than one measure of success.
†*p* Value from logistic regression.

labor parameters. Of the placebo patients who could not be evaluated for labor parameters, two had delays from pessary removal to initiation of induction of labor, one failed to go into labor after an extended induction and had a primary cesarean section, and three underwent serial attempts at induction with overnight rests between oxytocin administrations. The two active-agent patients who could not be evaluated had serial attempts at induction with overnight rests between oxytocin administration.

Statistical analysis was done with the Statistical Analysis System.[6] Categoric data were analyzed by either Pearson's χ^2 or logistic regression. Continuous variables were analyzed by analysis of variance and the F test.

Results

Demographic data (Table II), obstetric history (Table III), initial Bishop score (Table III), and primary indication for induction (Table IV) did not differ between the placebo and active-agent groups. This balance was not disturbed when the patients were stratified into those evaluable for cervical ripening and those evaluable for labor parameters or when these evaluable groups were substratified into multiparous and primiparous patients.

The primary outcome variable for this study was cervical ripening. Successful treatment outcome was defined as a change in Bishop score of ≥3, a Bishop score of ≥6, or vaginal delivery within 12 hours of pessary insertion. Table V lists several possible ways to gauge success in cervical ripening for evaluable patients. The results were unchanged when primiparous women were considered separately. When multiparous women were analyzed, only treatment outcome and onset of labor within 12 hours remained statistically different between the placebo and active agent.

Timing for labor events for evaluable patients is presented in Table VI. When primiparous patients were analyzed separately, only the time to rupture of membranes and the time to onset of labor remained statis-

Table VI. Summary of labor timing data for evaluable patients

	Placebo	*Active agent*	*p Value**
Time to rupture of membranes†			
Mean ± SD	23.9 ± 7.56 (n = 28)	15.2 ± 8.32 (n = 35)	0.0002
Range	15-46	3.5-42.8	
Time to onset of labor			
Mean ± SD	21.3 ± 7.46 (n = 26)	11.0 ± 9.76 (n = 34)	0.0002
Range	14-45	0.83-43.50	
Length of first stage of labor			
Mean ± SD	9.6 ± 5.80 (n = 21)	9.3 ± 5.38 (n = 29)	0.4348
Range	0.25-20.50	0.67-23.25	
Length of second stage of labor			
Mean ± SD	1.3 ± 1.08 (n = 20)	1.1 ± 1.25 (n = 30)	0.8012
Range	0.18-4.82	0.05-5.98	
Time to vaginal delivery			
Mean ± SD	32.3 ± 9.92 (n = 20)	20.9 ± 12.90 (n = 30)	0.0093
Range	18-75	5.0-55.7	

**p* Values from analysis of variance F test.
†Three patients with pessary insertion after membrane rupture not included.

tically different. For primiparous patients, the mean time to vaginal delivery with placebo was 32.9 hours and with active agent was 27.0 hours (p = 0.1633, analysis of variance F test). When multiparous patients were analyzed separately, in addition to the significant differences seen in the overall group, the mean length of the second stage of labor was significantly shorter with the active agent, 0.4 versus 0.9 hour with placebo (p = 0.0411, analysis of variance F test). For multiparous patients, the mean time to vaginal delivery was 29.7 hours with placebo and 14.0 hours with the active agent (p = 0.0016, analysis of variance F test).

Data for method of membrane rupture, type of analgesia in labor, method of delivery, and occurrence of episiotomy are listed in Table VII for evaluable patients. No statistically significant differences appeared when primiparous women were analyzed separately. The cesarean rate for primiparous women was 33% in both placebo and active-agent groups. When the multiparous women were analyzed separately, a statistically significant difference appeared in cesarean section rate, 33% for placebo versus 7% for active agent (p = 0.0100, Pearson's χ^2 test).

Data on oxytocin administration are given in Table VIII for evaluable patients. The results were unchanged when primiparous patients were analyzed separately. When multiparous patients were analyzed separately, the amount of oxytocin administered was no longer statistically different between the placebo and active agent. This may have been due to the small sample size, because all of the multiparous patients in the placebo group (n = 6) required oxytocin averaging 7.2 U total, whereas only 20% (n = 3) of the multiparous patients in the active-agent group required oxytocin averging 2.4 U total.

Three patients receiving the active agent had uterine hyperstimulation. No patient who received placebo had

Table VII. Summary of labor and delivery events for evaluable patients

	Placebo	*Active agent*	*p Value**
Rupture of membranes			
Spontaneous	7	12	0.7120
Amniotomy	22	25	
Unspecified	1	2	
Analgesia†			
Epidural	22	28	0.5790
Intramuscular	0	1	
Intravenous	11	13	
Local-pudendal	0	1	
Spinal	1	0	
Type of delivery			
Cesarean	13	9	0.2770
Low-forceps	5	4	
Mid-forceps	1	0	
Spontaneous	11	24	
Vacuum extraction	3	2	
Episiotomy			
No	15	26	0.1620
Yes	15	13	

**p* Values from Pearson's χ^2 test.
†Some patients received more than one type of analgesic.

uterine hyperstimulation. Uterine hyperstimulation was defined as uterine contractions lasting ≥2 minutes or a contraction frequency of five or more in 10 minutes with evidence that the fetus was not tolerating this contraction pattern, as demonstrated by late decelerations, severe variable decelerations, or fetal bradycardia.

The incidence of hyperstimulation in patients receiving the active agent was 7% in this study. These episodes occurred between 1 hour and 8 hours 50 minutes after pessary insertion. All resolved rapidly after removal of the pessary, and no further therapy was required. All episodes of hyperstimulation occurred after the onset of labor.

Table VIII. Oxytocin administration for evaluable patients

	Placebo	*Active agent*	*p Value**
Oxytocin administered			
No	0	18	0.0000
Yes	30	21	
Induction	26	10	
Augmentation†	4	11	
Amount of oxytocin administered (U)			
Mean ± SD	11.0 ± 8.49	5.7 ± 6.52	0.0213
Range	0.68-32.96	20-60	

**p* Value for categoric data from Pearson's χ^2 test. *p* Value for continuous data from one-way analysis of variance F test.
†Defined as the use of oxytocin to enhance uterine contractions already present.

Comment

The PGE_2 pessary, which released PGE_2 at a near-constant rate of approximately 1 mg/hr in vitro, tested in this study is effective in producing cervical ripening when compared with placebo. It has a distinct advantage over PGE_2 gel or suppositories in that it releases a small amount of PGE_2 over a prolonged period rather than the entire dose rapidly.

The active agent results in significantly more patients going into labor during its 12-hour administration period than placebo. These findings are observed for both primiparous and multiparous patients. When we look past cervical ripening to benefits from pessary use in the subsequent labor, we find that the active pessary shortens the time to rupture of membranes and to onset of labor. It also decreases the need to administer oxytocin.

Additional benefits achieved by multiparous women are a shortened second stage of labor, a shortened time to vaginal delivery, and a decreased cesarean section rate.

The primiparous women gained some additional benefits. The average total amount of oxytocin needed to be administered was cut almost in half, from 12.0 to 6.3 U. Twenty-five percent of the primiparous women given the active agent required no oxytocin, as opposed to all of those given placebo. Additionally, those patients receiving the active agent who also received oxytocin were more likely to require only augmentation (50% vs 8% with placebo). Thirty-eight percent of the primiparous women given the active agent went into labor during the 12-hour period of pessary insertion, whereas none of those patients on placebo did. The time from pessary insertion to vaginal delivery was not shortened by active agent (27.0 hours, range 7.2 to 55.7 hours, vs placebo 32.9 hours, range 18 to 53 hours). The failure to achieve statistical significance is probably due to the wide range of time taken to achieve vaginal delivery. This should not be surprising as Bishop[5] in reporting his scoring system cautioned against elective induction of primiparous patients, because, even in the presence of apparently favorable circumstances, the duration of labor is unpredictable.

Uterine contractions were frequently produced by the active pessary. Irregular contractions and frequent mild contractions compatible with the diagnosis of uterine irritability (not perceived as painful, ≤20 seconds' duration, every 1 to 2 minutes) were the most frequent patterns. Uterine hyperstimulation as defined previously had an incidence of 7% in this study among patients receiving PGE_2. Hyperstimulation resolved immediately on removal of the pessary and resulted in no emergency deliveries. The solid dosage form of the pessary allows for easy removal of the PGE_2 when compared with other dosage forms. All patients with hyperstimulation were multiparous women in labor at the time of the incident. Because of this risk of hyperstimulation that could occur at any time during the administration of the pessary, PGE_2 pessaries should be administered only to inpatients who are having continuous external electronic fetal monitoring and should be removed at the onset of labor as defined here.

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The resolution of chronic pelvic pain after normal laparoscopy findings

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Sixty patients with chronic pelvic pain were monitored after diagnostic laparoscopy had excluded obvious pelvic pathology. Six months after the procedure only two patients described their symptoms as unchanged or worse. Treatment should be limited to the minority of patients whose symptoms remain 6 months after laparoscopy. (Am J Obstet Gynecol 1992;166:835-6.)

Key words: Peritoneoscopy, abdominal pain

Chronic pelvic pain with no obvious pathologic cause is a common complaint in gynecology clinics and accounts for a large proportion of diagnostic laparoscopies. The findings on laparoscopy are grossly normal in ≤66% of cases.[1] A wide variety of causes have been postulated, including pelvic venous congestion in the majority of these women.

As to treatment of these women after normal laparoscopy findings, in a small prospective study Pearce et al.[2] found that nondirective psychologic counseling helped reduce pelvic pain, with a minority of the nontreatment control group being free from pain after 1 year. Our current management of these patients does not include such counseling; after normal laparoscopy findings, patients are discharged to their general practitioners. Our study was undertaken to assess whether this management is adequate.

Material and methods

Sixty premenopausal women with a median age of 29 years were recruited to the study after undergoing laparoscopies with normal findings. Seventy-five women with histories of chronic pelvic pain had initially had laparoscopies performed (by P.N.B. or E.M.S.). Fifteen patients were excluded on the basis of pathologic findings (8 cases of endometriosis, 5 pelvic adhesions, 1 appendicitis, and 1 ectopic pregnancy). All 60 women had histories of pelvic pain of ≥3 months' duration before the laparoscopy. The character of the pain varied, being described as unilateral by 27 (45%), bilateral by 12 (20%), and generalized by 21 (35%). Thirty-three women (55%) reported no relationship between their symptoms and their menstrual cycles, whereas 27 (45%) reported that their pain was cyclic in nature.

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All 60 women were reassured at a postoperative ward round that no gynecological abnormalities had been seen and were asked to attend outpatient follow-up appointments 6 weeks and 6 months after their laparoscopies. Recall of patients who did not initially attend follow-up appointments was done by mail and telephone. No detailed counseling was given at the follow-up appointments; patients were merely questioned about their symptoms and given general advice regarding simple analgesia.

Results

All 60 patients attended the 6-week follow-up appointment. Thirteen (22%) said they were pain free, 24 (40%) said their pain had diminished, and 23 (38%) said their pain was unchanged or worse.

Fifty-seven (95%) patients attended the 6-month follow-up appointment, with 33 (58%) reporting that they were pain free and 22 (39%) reporting that their pain had diminished. All 13 women who were pain free 6 weeks after the laparoscopy remained pain free 6 months after the procedure. None of these 55 patients believed that the pain had inconvenienced their lives to a significant extent in the 8 weeks before the 6-month appointment, and only three had taken simple analgesics to alleviate their symptoms. Two (4%) of the 57 patients described their symptoms as unchanged or worse. Both patients had consistently described their pain as generalized and unrelated to their menstrual cycles.

Comment

As in previous studies,[1] no diagnosis was made in this study after laparoscopy in the majority of women initially seen with chronic pelvic pain. However, from a pragmatic standpoint the findings we have presented suggest that the diagnosis itself is of limited importance.

Our study could be criticized for incorporating only a minimal attempt to quantify the subjects' pain. Nevertheless, the clear conclusion drawn from these results is that after a laparoscopy with normal findings, release

of the patient to the general practitioner is appropriate. Whatever the etiology of the pelvic pain, the symptoms of most patients improved or resolved. A psychologic component has frequently been suggested in women with chronic pelvic pain in the absence of pathology; presumably the reassurance derived from the results of the laparoscopy at least contributed to the improvement in symptoms. It would have been interesting to have compared this group with a similar group of patients who had not had laparoscopy to determine whether the laparoscope is truly therapeutic.

These findings were thus at variance with those of Pearce et al.[2] We suggest that treatment (including conseling) should concentrate on the small minority of patients whose symptoms remain 6 months after laparoscopy.

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Prostaglandin E_2 for induction of labor in patients with premature rupture of membranes at term

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OBJECTIVE: A prospective study comparing three management schemes for patients at term with premature rupture of membranes was performed.
STUDY DESIGN: One hundred forty patients were randomized to one of three study groups: prostaglandin E_2, placebo, or oxytocin. Patients randomized to prostaglandin E_2 and placebo received vaginal suppositories containing 3 mg prostaglandin E_2 or glycerin only, respectively; suppositories were administered in a double-blind fashion, on one or two occasions, 6 hours apart. Oxytocin was given only if labor was not established after 12 hours or to augment inadequate labor. In patients randomized to oxytocin labor was induced with intravenous oxytocin. The time interval to delivery, delivery outcome, and complications were analyzed.
RESULTS: Patients receiving prostaglandin E_2 were more likely to be in labor after one suppository and to be delivered without the addition of oxytocin when compared with placebo. The time interval to delivery was shorter with prostaglandin E_2 and oxytocin induction versus placebo ("expectant management"). The incidence of maternal infection was lowest in patients with labor induced by prostaglandin E_2. Although the overall cesarean section rate was low, there was a trend toward a lower rate with prostaglandin E_2 induction. No adverse effects were observed with prostaglandin E_2.
CONCLUSION: Prostaglandin E_2 can be used successfully to induce labor after premature rupture of membranes at term with greater ease of administration when compared with oxytocin. (Am J Obstet Gynecol 1992;166:836-43.)

Key words: Premature rupture of membranes, induction, prostaglandin

Management of the term gestation with spontaneous rupture of membranes before the onset of labor remains one of the most frustrating problems encountered by the practicing obstetrician. Its common occurrence and the considerable confusion that exists regarding the most appropriate course of management contribute to the frustration. An aggressive approach involving prompt administration of oxytocin to induce labor has been advocated in the past in an effort to reduce the significantly increased maternal and neonatal morbidity and mortality associated with infection[1]; however, oxytocin induction of labor in such patients has proved to be associated with an unacceptably high rate of cesarean delivery.[2-5] The vast majority of these operative deliveries are reported to be due to failed induction or lack of progress. A prolonged in-

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duction and labor with multiple pelvic examinations can result in intraamniotic infection, associated dysfunctional labor, cesarean section, and subsequent endometritis.[6, 7] The alternative, expectant management, has resulted in a cesarean section rate that is approximately half that noted with oxytocin-induced labor[2-5]; however, expectant management can result in significant delay before delivery. Evidence indicates that the rate of intrapartum fever increases with a latent period after rupture of membranes of >24 hours in term gestations. Perinatal mortality and neonatal infectious morbidity have been reported to increase as well after prolonged premature rupture of membranes.[8]

Prostaglandins have been used by a number of investigators to ripen the cervix and induce labor.[9] Intravaginal or intracervical prostaglandin E_2 (PGE_2) has been found to be efficacious in inducing labor at term,[10-17] with the majority of patients delivered without the aid of oxytocin, most within 24 hours. The success rate is dependent on dose of prostaglandin, location of administration, condition of the cervix at the onset of induction, and parity. The rates of cesarean section for failed induction have been low in these studies. Other reported advantages of the use of prostaglandin in comparison to oxytocin for labor induction include decreased blood loss at delivery, decreased use of analgesics in labor, a lower incidence of neonatal jaundice, and greater patient satisfaction. Patient side effects have been few and uterine hypertonus encountered infrequently.

Few reports of use of prostaglandins in patients with ruptured membranes have appeared in the literature.[18-22] These studies document successful induction of labor with various prostaglandin preparations. Several studies reported reduction in intravenous oxytocin, analgesia, and lowered cesarean section rates. This study was undertaken to compare the effectiveness of intravaginal PGE_2 for induction of term labor with oxytocin induction and expectant management in patients with spontaneous rupture of membranes before the onset of labor.

Material and methods

The study was performed at the University of California, Irvine Medical Center and Long Beach Memorial Medical Center. Patients, recruited from both the private and clinic obstetric services, gave their informed consent. This study was approved by the human subjects committees of both institutions. Eligible patients included those at term (>36 weeks) with spontaneous rupture of membranes who presented before the onset of labor. All patients had singleton pregnancies with vertex presentations and no evidence of intrauterine infection or fetal distress. Rupture of membranes was documented by sterile speculum examination with positive ferning and Nitrazine tests. A digital cervical examination was not carried out until labor or oxytocin augmentation began. The cervix was observed on the initial sterile speculum examination and patients with advanced dilatation (≥3 cm) were excluded. Uterine activity of two or more contractions per 10 minute intervals at presentation was considered evidence of labor and resulted in exclusion. Patients who met all of the above criteria were excluded only if they refused to participate.

Eligible patients were randomly assigned to one of three study groups: PGE_2, placebo, or oxytocin. The randomization was performed by selecting the study group next on a random list maintained by the pharmacy personnel. Each time a patient was enrolled, the pharmacy was contacted and the labor and delivery staff informed if the patient was to receive oxytocin or suppository (PGE_2 or placebo).

Patients randomized to the oxytocin group immediately received intravenous oxytocin. The protocol for oxytocin induction in effect at the time of the study called for initiation of oxytocin by infusion pump at 3 mU/min and incremental increases (1 mU/min every 20 minutes) until adequate uterine activity was achieved. The protocol was consistently applied at both institutions and for all study groups. Internal monitors were used whenever the uterine contraction information provided by the external tocodynamometer was thought to be inadequate, fetal distress was suspected, or progress in labor deemed to be slow. Adequate uterine activity was defined as three uterine contractions of at least 50 mm Hg in intensity per 10-minute intervals.

Patients randomized to the PGE_2 and placebo groups received a vaginal suppository. PGE_2 suppositories were prepared by thoroughly mixing 20 mg PGE_2 suppositories in a glycerin base and creating individual suppositories containing 3 mg PGE_2. Placebo suppositories contained only glycerin. The suppositories were indistinguishable and given in a double-blind fashion. The code was not broken until data evaluation was begun after completion of the study.

The vaginal suppositories were carefully placed in the posterior fornix with a sterile glove. Patients receiving suppositories were reevaluated in 6 hours. Patients without regular uterine contractions, defined as more than two perceived uterine contractions per 10-minute interval, received a second suppository, the content of which was the same as the first. After an additional 6 hours, the patients were evaluated again, and those without regular uterine contractions received intravenous oxytocin, as noted. No digital cervical examinations were performed until patients were considered to be in labor or oxytocin was begun. Oxytocin for augmentation was used in patients with inadequate labor patterns and failing progress. Internal pressure catheters were used frequently to assess uterine activity when progress of labor was deemed to be slow. Deci-

Table I. Comparison of demographic and labor characteristics

	PGE_2	Oxytocin	Placebo	p Value
No.	40	55	45	
Maternal age (yr)	26.3	25.4	25.6	NS
Nulliparous (%)	47	53	52	NS
Duration of rupture of membranes on admission (hr)	8.34	10.96	7.69	NS
Use of oxytocin (%)	37.5	100	64.4	<0.05*
Highest oxytocin (mU/min)	7.8	7.4	6.6	NS
Second suppository (%)	47.5	—	71.1	0.02*
Time interval to delivery† (hr)	16.3	13.9	21.2	<0.05‡
Time interval to vaginal delivery† (hr)	15.3	12.8	19.4	<0.05‡

NS, Not significant.
*PGE_2 versus placebo.
†Mean time interval from intervention.
‡PGE_2 and oxytocin versus placebo.

sions regarding the course of labor were made by the resident physicians and attending staff on the basis of standard obstetric practice. Failure to progress resulting in delivery by cesarean section included patients with prolonged latent phase (>18 hours) and lack of dilatation or descent in active phase (<1 cm in 2 to 3 hours). Chorioamnionitis was diagnosed if maternal temperature exceeded 38° C during labor without evidence of other sources of fever. Antibiotics were initiated when the diagnosis was made. Endometritis was diagnosed when maternal temperature exceeded 38° C on two occasions or 38.5° C on one occasion after the first 24 hours post partum when uterine tenderness was present and other sources of fever were excluded. Prophylactic antibiotics were used routinely at the time of cesarean section. Neonates were admitted to the neonatal intensive care unit to rule out sepsis when prolonged rupture of membranes (>24 hours) and maternal fever during labor were present. Maternal records were reviewed to assess the duration of ruptured membranes, need for oxytocin, highest dose of oxytocin, time interval from intervention (oxytocin induction or placement of first suppository) to delivery, mode of delivery, maternal side effects and infectious morbidity, fetal effects, and neonatal morbidity. Statistical analysis was applied using χ^2, Fisher exact, and Student *t* test where appropriate.

Results

One hundred forty-three patients were randomized. Three patients, two in the placebo and one in the PGE_2 group, were excluded because of noncompliance with the study protocol. No significant differences in mean maternal age, parity, or duration of rupture of membranes on admission, were found among the three groups (Table I). The number of patients requiring oxytocin for induction or augmentation was significantly less with PGE_2 compared with placebo. The mean highest dose of oxytocin administered did not differ among the three groups. Of patients receiving suppositories, 47.5% in the PGE_2 group versus 71.1% in the placebo group required a second suppository after 6 hours ($p = 0.02$). Thus 52.5% of patients with labor induced with PGE_2 were having regular uterine contractions within 6 hours of receiving a single dose. The mean time interval to delivery with PGE_2 induction (16.3 ± 9.4 hours) and oxytocin induction (13.9 ± 7.7 hours) was significantly less than with placebo (21.2 ± 9.9 hours, $p < 0.05$). The cumulative number of patients delivered at ≤12 and ≤18 hours was significantly greater with PGE_2 and oxytocin induction than with placebo ($p < 0.01$) (Fig. 1). Among patients delivered vaginally, the mean time interval to delivery was significantly less with PGE_2 induction (15.3 ± 8.7 hours) and oxytocin induction (12.8 ± 7.3 hours) than with placebo (19.4 ± 8.7 hours, $p < 0.05$). The incidence of maternal infection requiring antibiotic treatment, which includes chorioamnionitis and endometritis, was lowest with PGE_2 (Fig. 2). A trend toward a lower incidence of cesarean section and cesarean section for failure to progress was seen in the PGE_2 induction group; however, this did not reach statistical significance (Fig. 3). There were no apparent differences between the groups in the distribution of cesarean sections for failure to progress in latent phase versus active phase. However, the number of cesarean sections performed is too small to allow statistical analysis.

Maternal side effects were minimal. Three patients who received suppositories, one in the PGE_2 and two in the placebo group, complained of nausea with or without vomiting. Two patients in the placebo group required subcutaneous terbutaline for treatment of

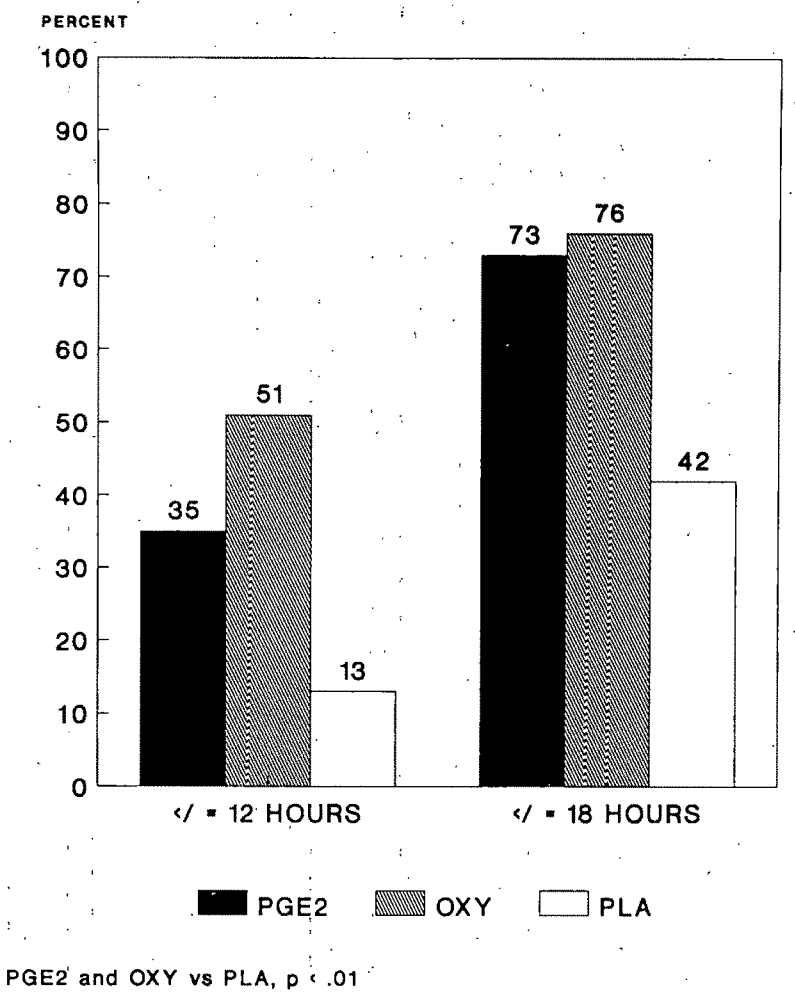


Fig. 1. Cumulative number of patients delivered.

prolonged decelerations of the fetal heart rate caused by hyperstimulation. There were no instances of hyperstimulation necessitating tocolytics in either the PGE_2 or the oxytocin group. One patient in the oxytocin group was delivered vaginally of an infant with a 5-minute Apgar score <7 after a prolonged deceleration. Seven neonates were admitted to the neonatal intensive care unit to rule out sepsis, two from the PGE_2 group, two from the oxytocin group, and three from the placebo group. In none of the neonates was sepsis documented and all had uneventful neonatal hospital stays.

The data for nulliparous patients and patients with at least one prior delivery (para ≥1) were analyzed separately (Tables II and III). All the trends previously noted in the overall population were evident for both the nulliparous and parous patients; however, with the smaller size of the groups not all comparisons reached statistical significance. The parous patients receiving PGE_2 for induction appeared to do particularly well. Only 21% of these patients required oxytocin and nearly 90% were delivered within 18 hours. This group had no cesarean sections, compared with four cesarean sections for failed progress among parous patients who received oxytocin induction.

When the results were stratified by hospital, there were no differences observed in the above noted trends.

Comment

The use of prostaglandins for cervical ripening and labor induction has been investigated in numerous randomized trials. Efficacy in both nulliparous and parous patients has been documented with few maternal side effects and good neonatal outcome.[10] Successful induction of labor and delivery without the addition of oxytocin has been reported in 60% to 90% of parous patients and 30% to 70% of nulliparous patients with intact membranes.[11-17] Few studies have considered patients with ruptured membranes. Hauth et al.[18] re-

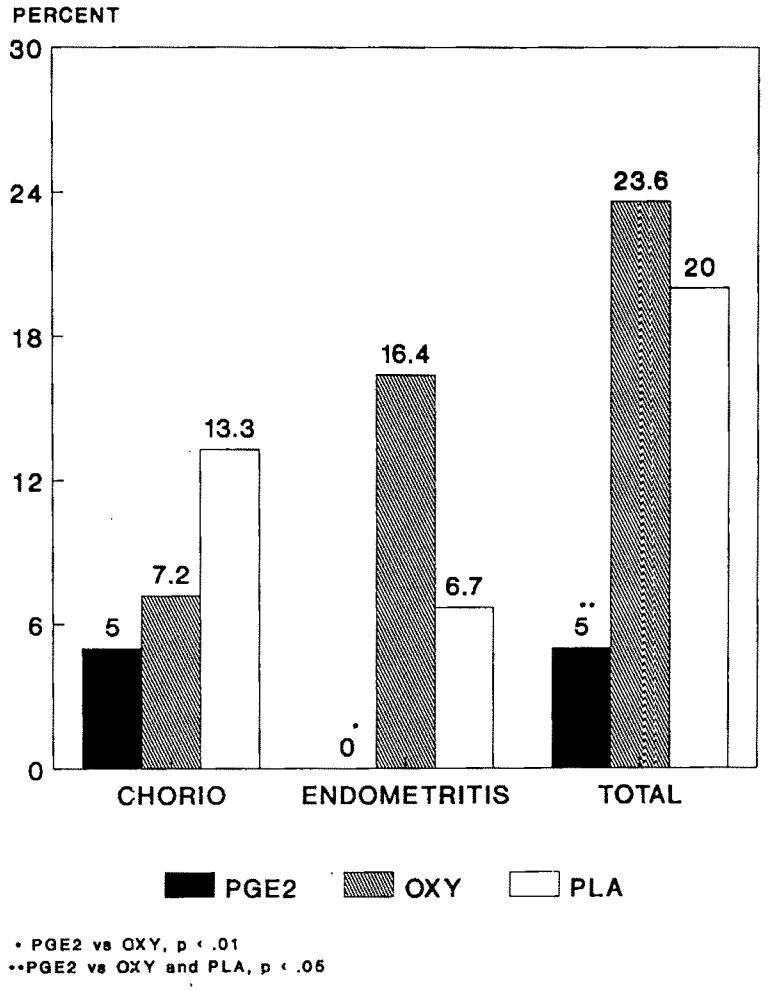


Fig. 2. Maternal infectious morbidity.

Table II. Nulliparous patients

	PGE_2	Oxytocin	Placebo
No.	21	29	21
Maternal age (yr)	24.0	22.4	22.4
Duration of rupture of membranes on admission (hr)	6.86	9.87	10.13
Use of oxytocin (%)	52.4	100	66.7
Interval to delivery (hr)	19.76	16.43	24.36*
Delivery ≤12 hr (%)	23.8	34.5	9.5*
Delivery ≤18 hr (%)	57.1	65.5	28.5*
Interval to vaginal delivery (hr)	18.10	15.26	22.75*
Maternal infection (%)	9.5†	34.5	33.3
Cesarean section (%)	14.3	20.7	19.0
Cesarean section (failure to progress) (%)	14.3	17.2	19.0

*Placebo versus oxytocin, $p < 0.05$.
†PGE_2 versus oxytocin, $p < 0.05$.

ported on 100 patients who were not in labor 3 hours after spontaneous rupture of membranes at 38 to 41 weeks. In their comparison of oral PGE_2 with intravenous oxytocin, prostaglandin was successful in initiating active labor in 88% of patients. The interval from rupture to delivery was shorter and the cesarean section rate lower in patients induced with prostaglandin. MacLennan and Green[19] reported successful induction of term labor in patients with spontaneous rupture of membranes receiving intravaginal prostaglandin $F_2\alpha$. Prins et al.[20] treated 20 patients between 32 and 43 weeks with premature rupture of membranes and an unfavorable cervix with 2.5 mg of PGE_2 intravaginally, with a 55% incidence of spontaneous labor and no failed inductions. Two published studies have compared intravaginal PGE_2 and intravenous oxytocin. Ma-

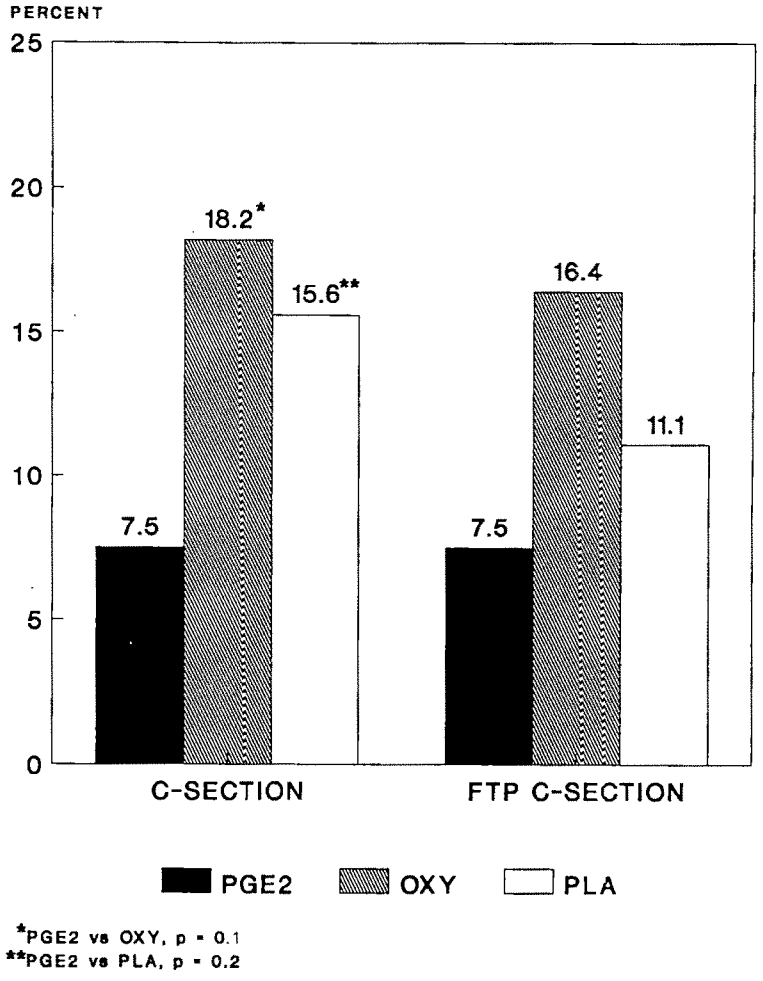


Fig. 3. Incidence of cesarean section.

Table III. Parous (para ≥1) patients

	PGE_2	*Oxytocin*	*Placebo*
No.	19	26	24
Maternal age (yr)	28.7	28.7	28.3
Duration of rupture of membranes on admission (hr)	9.77	12.17	5.55*
Use of oxytocin (%)	21.1†	100	62.5
Interval to delivery (hr)	12.5†	11.01	18.47*
Delivery ≤12 hr (%)	47.4†	69.2	16.7*
Delivery ≤18 hr (%)	89.5†	88.5	54.2*
Interval to vaginal delivery (hr)	12.55	10.31	16.76*
Maternal infection (%)	0	11.5	8.3
Cesarean section (%)	0	15.3	12.5
Cesarean section (failure to progress) (%)	0	15.3	4.2

*Placebo versus oxytocin, $p < 0.05$.
†PGE_2 versus placebo, $p < 0.05$.

gos et al.[21] reported equivalent outcomes when labor was stimulated with 3 mg PGE_2 pessaries and oxytocin after at least 4 hours of ruptured membranes in patients between 34 and 42 weeks. Ekman-Ordeberg et al.[22] randomized nulliparous term patients with premature rupture of membranes and unfavorable cervical status to receive either intravenous oxytocin or 4 mg PGE_2 administered intravaginally. One of 10 women receiving oxytocin was delivered vaginally within 24 hours versus six of 10 women after PGE_2 application.

This is the first report evaluating vaginally administered prostaglandin in patients with premature rupture of membranes at term in comparison with the two most commonly used methods of management, i.e., immediate induction with oxytocin and some period of expectant management before intervention with oxytocin. In the current study the placebo suppository group functions as an expectant management group,

as they received no mediation to initiate labor for 12 hours. This study included both nulliparous and parous patients of varying cervical status. We chose not to assess cervical Bishop score, as recent evidence has shown that infection rates increase when digital cervical examination is performed once membrane rupture has occurred.[6, 23] Additionally, we chose to assess the effect of PGE_2 in all patients to evaluate and compare its overall efficacy. Since patients with unfavorable cervical status are clearly the hardest in whom to successfully induce labor, it is possible that the differences might have been greater if only patients with unfavorable cervices had been included.

In this heterogeneous population, the use of PGE_2 significantly reduced the need for oxytocin and was highly successful in independently inducing labor, with nearly two thirds of patients not requiring oxytocin. Parous patients were even more likely to be successfully induced with prostaglandin alone (79%).

Although the latent period before the onset of labor in term patients with premature rupture of membranes may be prolonged, the duration of labor has been reported to be shorter with spontaneous labor after expectant management compared with oxytocin-induced labor.[4] Because the onset of labor is difficult to determine, we chose to evaluate the overall time interval from intervention (including onset of expectant management, i.e., placebo) to delivery. The current study demonstrates that both prostaglandin and oxytocin achieved equivalently shorter time intervals to successful vaginal delivery than did expectant management. We were unable to document any improvement in outcome with 12 hours of expectant management. This is in keeping with the results of a recent study comparing early versus delayed oxytocin induction of labor in 182 patients at term with spontaneous rupture of membranes in which no advantage to delay of induction was achieved with 24 hours of expectant management.[23]

The price that one pays for the shorter interval between rupture of membranes and delivery with oxytocin in most studies is a higher cesarean section rate. Compared with expectant management, oxytocin induction after premature rupture of membranes has been associated with rates of cesarean section ranging from 20% to 47%.[2-5, 22] The cesarean section rate in our population with oxytocin induction was relatively low compared with that in other reports (18% overall and 16% for failure to progress). However, the rate was even lower in the PGE_2 group (7.5%). Of the three types of management compared in the current study, the lowest cesarean section rate was achieved with vaginally administered PGE_2. Studies comparing oxytocin induction with expectant management have reported an increased incidence of maternal infections in patients with oxytocin induction. We also found a significantly increased rate of maternal infectious morbidity in the oxytocin group. This may be due at least in part to a tendency for more intervention, such as digital examinations and use of internal monitors in patients receiving oxytocin.

In this study the best combination of shortening the interval to delivery while keeping the cesarean section rate and maternal infection rate as low as possible was achieved with intravaginal PGE_2, followed by oxytocin when necessary.

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The cavitational ultrasonic surgical aspirator for cytoreduction in advanced ovarian cancer

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OBJECTIVE: The cavitational ultrasonic surgical aspirator is a newer surgical instrument that has been shown to be effective in tumor resection. The purpose of this study was to evaluate its usefulness for cytoreduction of advanced ovarian cancer.
STUDY DESIGN: Over a 24-month period patients with advanced ovarian cancer (stage IIIC and IV or bulky recurrent disease) were studied to determine the frequency of use of the cavitational ultrasonic surgical aspirator and the sites of tumor resection. The blood loss and operative time for patients who underwent surgery with the cavitational ultrasonic surgical aspirator were compared by Student *t* test with those of patients who did not undergo surgery with the cavitational ultrasonic surgical aspirator.
RESULTS: The cavitational ultrasonic surgical aspirator was utilized to effect optimal cytoreduction in 22 of 45 patients (48%). Of 52 sites of disease resected, 23 (44%) involved sites difficult to resect with standard operative techniques. These sites included the diaphragm, liver, spleen, and fixed retroperitoneal adenopathy. The cavitational ultrasonic surgical aspirator allowed resection from other sites, precluding procedures with potentially more morbidity. Of all 45 patients, 86% had cytoreduction to <2 cm and 44% to <0.5 cm. The blood loss and operative time were not different between cases in which the cavitational ultrasonic surgical aspirator was used and those in which it was not used.
CONCLUSION: The cavitational ultrasonic surgical aspirator appears to contribute to cytoreduction in patients with advanced ovarian cancer. (Am J Obstet Gynecol 1992;166:843-6.)

Key words: Ovarian cancer, cytoreduction, cavitational ultrasonic surgical aspirator

In advanced ovarian carcinoma, the volume of postoperative residual disease has been shown to significantly affect survival.[1] Furthermore, among the many factors that predict survival before chemotherapy initiation, this factor is the only one that is subject to modification. Aggressive surgical techniques have allowed the optimal debulking of postoperative residual disease in the majority of patients with advanced ovarian carcinoma.[2-4] Wharton and Herson[5] demonstrated that the survival of patients who required extensive surgical procedures was equal to that in those with easier cytoreduction. Although optimal debulking was originally defined as residual disease measuring 1.5 to 2 cm in greatest diameter, achieving even smaller residual disease appears to be beneficial.[1, 4]

The frequency of optimal cytoreduction varies in the literature and is a reflection of the aggressiveness of the surgical service. In addition to traditional surgical techniques, three newer instruments have been reported to be effective. These include the cavitational ultrasonic surgical aspirator,[6-8] the carbon dioxide laser,[9] and the argon beam coagulator.[10] The cavitational

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Table I. Sites and frequency of disease resected with the cavitational ultrasonic surgical aspirator in 22 patients

Bladder	4
Colon	5
Cul-de-sac	5
Diaphragm	7
Gallbladder	1
Gastrosplenic ligament	5
Liver	6
Paraaortic nodes	2
Pelvic nodes	2
Rectosigmoid colon	5
Small bowel	2
Spleen	6
Stomach	2
Total No. of sites	52

ultrasonic surgical aspirator has been reported effective in debulking tumor from the diaphragm,[6, 7] spleen,[6] liver,[8] and intestine[6, 8] without injury to these organs. It may offer an advantage over the other two methods because it selectively spares connective tissue and blood vessels. This article reports the frequency with which the cavitational ultrasonic surgical aspirator was used during cytoreductive surgery for ovarian carcinoma and its effectiveness in contributing to the cytoreductive effort.

Material and methods

All patients with advanced ovarian carcinoma (stage IIIC or IV or bulky recurrent disease) who were operated on for the purpose of cytoreduction at The University of Massachusetts Medical Center during a 24-month period, from Aug. 1, 1988, to Aug. 1, 1990, were included. Patients with ovarian cancer who underwent exploration for purposes other than cytoreduction, including second-look laparotomy and intestinal obstruction, were excluded.

In all cases the surgical procedure consisted of abdominal exploration and resection of the primary tumor and metastatic deposits by standard operative technique. In each case the cavitational ultrasonic surgical aspirator was available and was used at the discretion of the surgeon if cytoreduction was not accomplished by standard operative techniques or if the cavitational ultrasonic surgical aspirator could be used with decreased morbidity. A cavitational ultrasonic surgical aspirator system 100 or 200 Macro-Dissector (Valleylab, Boulder, Colo.) with a frequency of 23,000 Hz was used to fragment and resect tumor. The amplitude of vibration controls the excursion of the instrument tip and the depth of tissue disruption. The amplitude setting most commonly used for tumor resection was 0.7 to 0.8 (210 to 240 μm). When the liver and spleen were resected, the amplitude was set at 0.4 to 0.5 (120 to 150 μm). The sites of tumor, operating time, blood loss, and postoperative residual disease were recorded. Statistical analysis was performed with a Student t test on Minitab Software (Minitab Inc., State College, Pa.).

Results

Forty-five patients with advanced ovarian disease including stage IIIC (n = 24), IV (n = 13), and recurrent disease >2 cm (n = 8) underwent cytoreductive surgery. The cavitational ultrasonic surgical aspirator was used in 22 (48%) (10 of 24 with stage IIIC, 9 of 13 with stage IV, and 3 of 8 with recurrent disease). The sites of disease that were resected with the cavitational ultrasonic surgical aspirator and the frequency with which these sites were resected are listed in Table I. Thirty-nine of the 45 patients (86%) had cytoreduction to <2 cm and 20 (48%) to <0.5 cm. The site of bulky residual disease was extraperitoneal in two of the six patients without optimal cytoreduction (supraclavicular node, n = 1, and lung, n = 1).

The diaphragm was the disease site where the cavitational ultrasonic surgical aspirator was most frequently used. Preresection tumor measurements varied from 5 × 5 × 5 to 5 × 8 × 10 cm. In the most advanced cases the liver was adherent to the diaphragm with tumor. In these cases the tumor between the liver and diaphragm was bluntly fractured, the liver was mobilized by transection of the falciform ligament, and the cavitational ultrasonic surgical aspirator was used to resect tumor from both the diaphragm and liver peritoneum. No pneumothoraces were demonstrated on postoperative chest radiographs.

Disease involving the liver parenchyma was resected in six patients. In five of the six the tumor was adequately cytoreduced. One patient, 25 years old, had multiple liver metastases measuring 8 cm in diameter, and only partial resection was accomplished. In this case the largest residual tumor measured 3 cm in diameter on a postoperative computed tomographic scan.

Six patients had bulky disease resected with the cavitational ultrasonic surgical aspirator that involved either the splenic hilum or the capsule. In one of these patients a splenic capsule laceration occurred because of operative retraction. This was controlled with conservative hemostatic techniques and observed without problems. In five cases tumor involving the gastrosplenic ligament was removed. During this same time period two patients underwent splenectomy by the gynecologic oncology service for cytoreduction.

The cavitational ultrasonic surgical aspirator was used for effective cytoreduction in the colon in 10 patients. This avoided additional intestinal resection. However, five of the 22 patients undergoing cavitational ultrasonic surgical aspirator did require intestinal resection. In two cases, because of previous pelvic or

Table II. Comparison of operative time and estimated blood loss

	Cavitational ultrasonic surgical aspirator (n = 22)	*No cavitational ultrasonic surgical aspirator (n = 23)*	*p Value*
Operative time (min, mean ± SD)	246 ± 89	266 ± 132	$p = 0.56$
Estimated blood loss (ml, mean ± SD)	1018 ± 803	897 ± 692	$p = 0.59$

abdominal radiation, a colostomy was performed. Eight of the 23 patients in whom the cavitational ultrasonic surgical aspirator was not used required intestinal resection to complete cytoreduction. Four of these patients required colostomy or ileostomy.

Four patients had resection of bladder tumor with the cavitational ultrasonic surgical aspirator. In two additional patients partial bladder resection was performed because tumor involving the bladder was adherent to the anterior abdominal wall.

There was no difference in the operative time or blood loss between those patients who underwent cavitational ultrasonic surgical aspirator resection and those treated by standard surgical techniques (Table II).

Comment

The cavitational ultrasonic surgical aspirator is a relatively new surgical instrument that uses ultrasonic vibration to fracture tissue that is high in water content. The instrument can be used to destroy tumor tissue because of its high water content and relative lack of collagen. It selectively spares connective tissue and prevents injury to bowel, blood vessels, or the peritoneum of the pelvis or diaphragm. The amplitude of vibration can be adjusted to suit the precision or rate of tissue destruction desired. Where there are smaller amounts of connective tissue, such as the liver or spleen, the amplitude of vibration must be decreased to avoid resection of normal organ parenchyma.

The goal of surgery in advanced and recurrent ovarian cancer is to minimize the amount of postoperative residual tumor. Certain tissues, including the liver and diaphragm, and cases of fixed retroperitoneal adenopathy are difficult to cytoreduce by standard operative techniques. Other sites of bulky disease, including the spleen, bladder, and small and large intestine, can be resected. However, each of these procedures is associated with its own morbidity. In the current study the cavitational ultrasonic surgical aspirator was found to be effective in contributing to cytoreduction in 48% of patients with advanced ovarian cancer. Of the 52 sites of disease resected, 23 (44%) involved either the diaphragm, liver, spleen, or fixed adenopathy. In the remaining 29 sites, the cavitational ultrasonic surgical aspirator precluded the need for more extensive surgery.

The frequency with which patients in this series had cytoreduction to <2 cm (86%) compares favorably with that of other reports in the literature.[2-4] Furthermore, 44% of patients had cytoreduction to <0.5 cm. On the basis of prior reports, such minimal residual disease should confer improved survival.[1, 4] However, longer follow-up will be required to determine the effect of such complete cytoreduction on the survival of our patient population.

One of the benefits of the cavitational ultrasonic surgical aspirator over laser techniques is that it selectively avoids injury to tissues with high connective tissue content. This has allowed it to be used safely on the intestine without reported injury.[6, 8, 11] However, in rare cases certain desmoplastic tumors with high collagen content may be difficult to resect.[8] Such fibrotic tissues can be optimally resected in combination with either standard operating techniques,[8] continued cavitational ultrasonic surgical aspirator resection,[12] or laser techniques.[9] Only one patient in the current series had such a desmoplastic tumor. This patient had optimal cytoreduction by a combination of the cavitational ultrasonic surgical aspirator with standard techniques.

One of the criticisms of the cavitational ultrasonic surgical aspirator has been that the dissection is tedious and operating time increased.[8, 11] Adelson et al.[6] reported its use in 10 patients with intraabdominal tumors with a mean operating time of 49 minutes. However, operating time may be offset if extensive dissection and reconstruction required by standard techniques can be avoided. For example, if the cavitational ultrasonic surgical aspirator is used to resect tumor involving the sigmoid colon, this should be compared with the time to resect the sigmoid colon and the time to complete either intestinal reanastomosis or diversion. In our study the operating room time in those patients in whom the cavitational ultrasonic surgical aspirator was used was slightly shorter than but not statistically different from that in patients in whom it was not used.

Few studies have examined operative blood loss during cavitational ultrasonic surgical aspirator surgery. Deppe et al.,[8] reporting their results in 11 women with ovarian cancer, noted an apparent decrease in blood loss.[8] In our study the mean operative blood loss was 1018 ml and was not statistically different from that in patients in whom the cavitational ultrasonic surgical aspirator was not used.

In this study the cavitational ultrasonic surgical aspirator appeared to contribute to the cytoreductive effort, because it was used by the surgeon in nearly half of the cases after standard operative techniques already had been used for cytoreduction. However, the reader should be aware that proof of the cavitational ultrasonic surgical aspirator's efficacy would require a randomized study where patients would by chance undergo completion of their cytoreduction with the cavitational ultrasonic surgical aspirator or be left with bulkier residual disease. Additionally, subsequent long-term follow-up is needed to prove a survival benefit to those patients who underwent cavitational ultrasonic surgical aspirator–assisted cytoreduction. Because cytoreductive surgery, although based on retrospective nonrandomized analyses, is so basic to our current management of ovarian cancer, such a study is unlikely to be completed.

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Does oxytocin augmentation increase perinatal risk in primigravid labor?

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To assess the influence of high-dose oxytocin augmentation of spontaneous labor, a consecutive series of 30,874 primigravid term deliveries were analyzed for adverse perinatal outcome. In spite of a longer mean duration of labor, the frequencies of asphyxial perinatal death, neonatal seizures, and abnormal neonatal neurologic behavior were not significantly increased in 14,119 (45%) oxytocin-treated patients. There was no case of uterine rupture in any primigravid labor during the study. These results from 13 years of clinical practice provide reassurance about maternal and fetal safety if oxytocin is used as part of a protocol of active management to correct dystocia when spontaneous primigravid labor with vertex presentation fails to progress. (Am J Obstet Gynecol 1992;166:847-50.)

Key words: High-dose oxytocin augmentation, active management of labor, adverse perinatal outcome

It has been suggested that dystocia is the primary cause of the rising international rates of cesarean section in first labors.[1-3] Early diagnosis and correction of dystocia in primigravid women have been shown to effectively counter this rising trend in both Europe[4-6] and North America.[7,8] High-dose oxytocin administration forms an essential part of programs of active augmentation by improving uterine contractility and preventing prolonged labor.[9] It has, however, been suggested that the price to be paid for more efficient uterine activity may be an increased risk of perinatal asphyxia resulting from myometrial hyperstimulation.[10-12]

At the National Maternity Hospital in Dublin, a standard regimen of high-dose (6 to 40 mU/min) intravenous oxytocin therapy has been used in slowly progressing primigravid labor for >20 years. The fact that no case of uterine rupture has resulted from this regimen underlines its maternal safety in relation to augmented primigravid labor.[13] To reassess the influence of oxytocin on the incidence of perinatal asphyxia, we analyzed our experience with oxytocin augmentation in spontaneous primigravid labor at term. Only primigravid women were included because of the well-documented risks of uterine hyperstimulation in multigravid women.[14,15]

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Methods

An analysis of 35,726 consecutive primigravid deliveries was undertaken at the National Maternity Hospital during the period January 1976 to December 1988. Excluded from analysis were multiple pregnancies (n = 330), elective (prelabor) cesarean sections (n = 494), labor induced by medical or surgical methods (n = 1762), breech and other malpresentations (n = 751), and deliveries <37 completed weeks' gestation (n = 1515). All births occurred in our hospital. Data on the augmentation of labor, perinatal deaths, and abnormal neurologic behavior were collected prospectively during the period and were collated in the hospital's Annual Clinical Reports (1976 through 1988).[13] Individual patient records were reviewed where appropriate.

Postmortem examinations were carried out on all term perinatal deaths during the 13 years. Asphyxial perinatal death was defined according to autopsy findings in infants who died during labor or in the first week of life but excluded deaths associated with cord prolapse. Abnormal neurologic behavior was defined as a syndrome of abnormal tone and primitive reflexes with evidence of cerebral irritation manifested by irritability or motor seizures[16]; asphyxial seizures were diagnosed in the presence of any myoclonic, multifocal clonic, generalized tonic, or focal clonic activity within the first week of life in the absence of a metabolic or infective cause.[17]

Primigravid labor was managed along uniform and clearly defined guidelines throughout the period studied.[15] Diagnosis of labor was based on the presence of uterine contractions supported by spontaneous rupture

Table I. Adverse perinatal outcome related to oxytocin augmentation in 30,874 consecutive spontaneous primigravid labors

	Oxytocin augmentation (n = 14,119)	*No oxytocin (n = 16,755)*	*p Value**
Asphyxial death	24 (1.7/1000)	28 (1.7/1000)	0.94, NS
Asphyxial seizures	31 (2.2/1000)	21 (1.3/1000)	0.06, NS
Neurologic abnormality without seizures	22 (1.7/1000)	20 (1.3/1000)	0.48, NS

*χ^2 Test.

of the membranes or passage of blood-stained mucus vaginally and effacement or dilatation of the cervix on pelvic examination. Oxytocin augmentation was defined as stimulation after the spontaneous onset of labor and was initiated in primigravid women if, within 3 hours of admission, cervical dilatation had not progressed by 1 cm/hr. Oxytocin was administered by infusion of 10 U/L of 5% dextrose solution, commencing at 6 mU/min and increasing incrementally every 15 minutes to a maximum rate of 40 mU/min. Oxytocin usage was confined to primigravid women with singleton pregnancies at term with vertex presentations and with no evidence of fetal distress; if a fetal heart rate abnormality or meconium-stained amniotic fluid was detected, oxytocin was withheld until normal fetal pH status was established by scalp blood sampling. Fetal surveillance was generally performed by intermittent auscultation.[18] Continuous electronic fetal monitoring was carried out on a selective basis when indicated by abnormal fetal heart rate on auscultation, meconium-stained or absent amniotic fluid after amniotomy, or preexisting conditions such as fetal growth retardation or antepartum hemorrhage.

At birth, asphyxial morbidity was assessed by (1) Apgar scores <7 at 1 minute, (2) when performed, cord venous pH values <7.2, and (3) pediatric follow-up of all babies showing abnormal neurologic behavior, with or without seizure activity, during the first week of life; cord pH was routinely determined in infants requiring resuscitation at birth only during the last 5 years of the study period, and so the numbers for analysis of this parameter are smaller.

Statistical analysis was performed with the Student *t*, χ^2, and Fisher exact tests, of significance at the 5% level.

Results

The overall hospital perinatal mortality rate (including stillbirths and first-week neonatal deaths after 28 weeks' gestation) among primigravid women during the period was 14.6 per 1000 (10.1/1000 when lethal malformations were excluded). The overall primigravid incidences of asphyxial neonatal seizures and abnormal neonatal neurologic results were 2.0 per 1000 and 1.4 per 1000, respectively. A total of 35,726 primigravid women were delivered during the 13 years studied, 930 (2.6%) of whom labored for ≥12 hours.

After 4852 exclusions were made on the basis of the criteria already mentioned, the study group of 30,874 primigravid women remained who entered labor spontaneously at term with singleton live pregnancies and vertex presentations. Of these, 14,119 (45%) received oxytocin to augment labor that was progressing slowly, as defined by a rate of cervical dilatation of <1 cm/hr. The incidence of intrapartum cesarean section was 3.1%; the incidence of instrumental delivery was 13.9%. There was no case of uterine rupture among the patients studied.

In total, there were 52 (0.17%) asphyxial perinatal deaths, 23 stillbirths, and 29 neonatal deaths. Ninety-four (0.3%) infants showed abnormal neurologic behavior; 52 (0.16%) of them had seizures associated with evidence of asphyxia. Oxtyocin augmentation did not influence the incidence of these complications (Table I).

Among those primigravid women with an adverse perinatal outcome, patients with oxytocin augmentation did not differ significantly in terms of maternal or gestational age from those not given oxytocin (Table II). Not surprisingly, however, mean duration of labor was significantly longer in association with augmentation and mean birth weight was higher among the oxytocin-treated patients with adverse outcomes. The duration of augmentation was similar, regardless of the type of adverse outcome, with mean total administered oxytocin dosages of 8 U.

Cord venous pH estimations were performed in 80 (65%) of 123 live born infants who had an adverse outcome (Table III). In 57 (71%) cases the value was low (pH <7.20). The proportions of infants with low cord pH values were not different between the augmented and nonaugmented groups. One hundred four (85%) infants had 1-minute Apgar scores <7; a greater proprtion (54/59, 92% vs 50/64, 78%) of these low scores associated with adverse outcomes followed nonaugmented labor.

Comment

The purpose of this study was to assess whether adverse perinatal outcome, specifically asphyxial perinatal death and abnormal neonatal neurologic behavior, with or without early seizures, was more common after the use of oxytocin to augment spontaneous labor in sin-

Table II. Comparisons of maternal age, gestational age, birth weight, and duration of labor in primigravid women who experienced adverse perinatal outcomes related to oxytocin augmentation of spontaneous labor

	Oxytocin augmentation	*No oxytocin*	*p Value**
Age (yr)			
Asphyxial deaths (n = 52)	24.5 ± 5.4	23.6 ± 4.6	NS
Asphyxial seizures (n = 52)	23.2 ± 3.7	25.1 ± 4.3	NS
Neurologic abnormality without seizures (n = 42)	24.0 ± 6.1	25.9 ± 4.7	NS
Gestational age (wk)			
Asphyxial death (n = 52)	40.6 ± 1.5	40.2 ± 1.6	NS
Asphyxial seizures (n = 52)	40.5 ± 1.5	40.7 ± 1.6	NS
Neurologic abnormality without seizures (n = 42)	41.1 ± 1.4	40.7 ± 1.0	NS
Birth weight (gm)			
Asphyxial death (n = 52)	3508 ± 533	3133 ± 506	$p < 0.05$
Asphyxial seizures (n = 52)	3280 ± 362	3143 ± 397	NS
Neurologic abnormality without seizures (n = 42)	3473 ± 624	3299 ± 507	NS
Duration of labor (hr)			
Asyphyxial death (n = 52)	8.8 ± 2.6	3.3 ± 2.1	$p < 0.02$
Asphyxial seizures (n = 52)	8.5 ± 3.6	2.8 ± 1.7	$p < 0.02$
Neurologic abnormality without seizures (n = 42)	8.6 ± 2.5	3.4 ± 2.2	$p < 0.02$

NS, Not significant.
*Student t test.

Table III. Comparison of low cord venous pH values (<7.2) and low Apgar scores (<7 at 1 minute) in primigravid women with adverse perinatal outcomes related to oxytocin augmentation of spontaneous labor

	Oxytocin augmentation	*No oxytocin*	*p Value**
Venous cord pH <7.2			
Asphyxial death (n = 16)	5/8 (63%)	6/8 (75%)	1.0, NS
Asphyxial seizures (n = 32)	10/14 (71%)	11/18 (61%)	0.71, NS
Neurologic abnormality without seizures (n = 35)	17/20 (85%)	10/15 (67%)	0.25, NS
Apgar score <7 at 1 min			
Asphyxial neonatal deaths (n = 29)	11/11 (100%)	17/18 (94%)	1.0, NS
Asphyxial seizures (n = 52)	25/31 (81%)	20/21 (99%)	0.22, NS
Neurologic abnormality without seizures (n = 42)	14/22 (64%)	17/20 (85%)	0.12, NS

NS, Not significant.
*Fisher's exact test.

gleton pregnancies at term with cephalic presentations. These results show that high-dose oxytocin acceleration does not increase fetal risk as measured by these adverse outcome variables. The protocol of labor management and fetal surveillance did not alter throughout the study period.

Although this investigation is retrospective and the statistical power of analyzing the small abnormal subgroups is somewhat reduced, nevertheless untreated patients who experienced bad outcomes did not differ from the oxytocin-treated group in any respect, apart from their more efficient uterine activity, shorter labors, and apparently smaller babies (birth weight being significantly different only in cases of perinatal death). Therefore our results are reassuring when viewed in association with evidence that cord arterial pH and P_{CO_2} values are unaffected by high-dose oxytocin stimulation.[19] In addition, we can confirm that oxytocin is completely safe in primigravid women in terms of the risk of uterine rupture.

The duration of primigravid labor at term is directly correlated with birth weight,[20] and our finding in complicated pregnancies that the duration of labors augmented with oxytocin was longer merely reflects the restriction of oxytocin usage to primigravid women in whom the progress of cervical dilatation was delayed. Indeed, further prolongation of such labors, were oxytocin withheld, might have been expected to increase, rather than reduce, the likelihood of fetal hypoxia.[21] Given that oxytocin-stimulated labors lasted longer and were thus potentially more hypoxic, the absence of a preponderance of perinatal complications in this group

further emphasizes the safety of the active management protocol used. Slow progress of cervical dilatation during the first stage of labor is the most reliable clinical indication of dystocia in primigravid women. A consequence of the avoidance or inadequate administration of oxytocin in these circumstances is an inevitable acceptance of a greater incidence of cesarean delivery in such patients. Our data suggest that such an approach cannot be justified on the grounds of potentially increased fetal jeopardy in augmented labors. When suboptimal dosages of oxytocin are used, cervical dilatation may be stimulated in some subjects, but cesarean delivery rates up to sixfold higher have resulted from uncorrected dystocia, often after labors lasting considerably longer than our 12-hour maximum.[22]

Our analysis of a very large number of first labors managed with a standard intrapartum regimen includes an asphyxial mortality rate of <2 per 1000 among nulliparous women during the period studied. By reflecting the practice of a large obstetric hospital over many years, rather than a restricted research context, it serves to emphasize that oxytocin used in clearly defined clinical circumstances in primigravid labor does not increase fetal hazards but instead acts as the keystone for the early correction of dystocia and helps to maintain a low incidence of primary cesarean delivery without additional fetal jeopardy.

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The frequency of breech presentation by gestational age at birth: A large population-based study

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From a study of birth records, breech presentation at delivery for each gestational age was found to be less frequent as compared with other reports about antenatal ultrasonographic examination. Selection bias or the effect of labor may account for the observed difference. (AM J OBSTET GYNECOL 1992;166:851-2.)

Key words: Breech presentation, gestational age, ultrasonography

Breech delivery has always been a subject of great interest, in part because of the associated increased perinatal mortality and morbidity. Although the greatest cause of the adverse outcome associated with breech delivery is low birth weight, congenital malformations also contribute to the increased morbidity and mortality risk. Recent reports concerning the frequency of breech presentation by gestational age have been derived by antenatal ultrasonographic examination.[1, 2] We undertook this study to examine this relationship with a large, population-based data set of birth records.

Material and methods

We examined computerized birth certificate tapes for 136,199 births occurring during 1986 and 1987 in Washington State. For the purpose of this study, we excluded multiple births because they are associated with an excess abnormal fetal presentation. To most accurately describe the overall frequency of breech presentation by gestational age, we did not exclude cases with fetal or uterine anomalies. Information was missing in 9028 cases (7.0%), leaving 127,171 births for analysis. Accuracy of information in the Washington State Birth Record is excellent, with an error rate of <4% as determined by comparison with hospital records.

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Results

The frequency of breech presentation was highest at the earliest gestational ages (Table I). In Washington State 20.6% of deliveries at 25 to 26 weeks were complicated by breech presentation. The frequency of breech presentation dropped rapidly after 28 to 30 weeks to a nadir of 1.7% after 40 weeks. After comparison with that in other series as determined by ultrasonography, the prevalence of breech presentation in Washington State was less for every gestational age category.

Table I. Breech presentation by gestational age at delivery

Duration of gestation (wk)	*Breech presentation (%)*		
	Scheer and Nubar[1] (n = 2276)	*Hill[2] (n = 4024)*	*Washington State (n = 127,171)*
25-26	31.8*	NA	20.6
27-28	24.9	24.4	21.7
29-30	16.0	18.7*	11.5
31-32	12.7*	13.3*	7.4
33-34	10.8*	9.4*	6.3
35-36	7.0	6.8	4.9
37-38	7.0*	3.7†	3.6
39-40	6.1*	NA	2.6
>40	NA	NA	1.7

NA, Not available.
*Two-sided p value <0.05 (χ^2 test comparing each study with Washington State).
†Reported for 37 to 40 weeks.

Comment

Although gestational age is the single greatest determinant of fetal presentation, other causes have been ascribed, including uterine relaxation as a secondary effect of parity, hydramnios, uterine anomalies (including fibroids), and abnormal placental implantation.

Scheer and Nubar[1] reviewed ultrasonography findings on 2276 patients in the second half of pregnancy at the Boston Hospital for Women for a 12-month period during 1974 and 1975. There were multiple indications for most examinations; rarely was ultrasonography performed only for ascertainment of fetal lie. Their findings were similar to those of Hill,[2] who reviewed 4024 ultrasonographic examinations performed in the second and third trimesters at the Mayo Clinic during 1982 and 1983. Information was used from the first visit only, and cases with twins, congenital anomalies, polyhydramnios, oligohydramnios, and uterine anomalies were excluded.

Our study differs in several important respects. The frequencies presented are derived from population-based data and are less subject to potential selection biases than hospital-based studies of patients seen at referral centers. Although the data base used for this study was large, it still may not be applicable to other populations with differing demographic and reproductive characteristics. Seven percent of the birth records had missing information for presentation at delivery; however, unless the majority of the missing cases were actually breech, the frequencies would remain relatively unchanged.

Our finding of less frequent breech presentation at each gestational age varies somewhat from the findings of other investigators. It is especially evident from 29 to 32 weeks, where a difference of >5% is found between this study and others. We believe that our data represent a more accurate description of this relationship, perhaps because information from delivery rather than from antenatal ultrasonography was used. It is also possible that labor may have an effect on the final presentation of the fetus before birth. Alternatively, selection bias may account for the higher frequency of breech presentation at all gestational ages, as reported by other authors.

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Ovarian carcinoma of low malignant potential, infertility, and induction of ovulation—Is there a link?

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Three cases of ovarian carcinoma of low malignant potential associated with infertility and ovulation induction are reported. The natural history of ovarian epithelial tumors is possibly being interrupted with earlier intervention and diagnosis in patients who may have presented 10 to 15 years later with disseminated ovarian carcinoma. (AM J OBSTET GYNECOL 1992;166:853-4.)

Key words: Ovarian carcinoma, infertility, ovulation induction

There appears to be an increasing incidence of borderline tumors of the ovary in young women. We observed an association of these ovarian tumors with a history of infertility and subsequent induction of ovulation. Three patients <35 years old presenting to this institution with the diagnosis of ovarian carcinoma of low malignant potential had histories of infertility, infertility management including laparoscopy, induction of ovulation with menotropins (Pergonal, Serono Laboratories, Randolph, Mass.), and serial ultrasonography to evaluate follicular size, and pregnancy.

Fischel and Jackson[1] postulated the potential risk of ovarian, endometrial, and breast carcinoma from excessive ovarian stimulation. There have been reports in the British and European literature of seven cases of ovarian carcinoma associated with ovulation induction.[2] These cases were all associated with clomiphene citrate induction of ovulation. Six of these seven patients received clomiphene for >1 year and one received clomiphene plus Pergonal.

To our knowledge there has not been a report of ovarian carcinoma associated with induction of ovulation in the American literature.

All the patients in our series received Pergonal therapy for induction of ovulation, and serous ovarian tumors of low malignant potential developed in all.

Case reports

Case 1. A 32-year-old woman with a long history of primary infertility was enrolled in the in vitro fertilization program and received two cycles of Pergonal, resulting in a successful intrauterine pregnancy with the second cycle. She underwent cesarean section and was noted to have excrescences on the right ovary. Biopsies revealed a serous adenocarcinoma of low malignant potential. Elective surgical staging and right salpingo-oophorectomy confirmed the diagnosis.

Case 2. A 25-year-old woman with a 2-year history of primary infertility was accepted into the in vitro fertilization program and had three cycles of induction of ovulation with Pergonal. A successful intrauterine pregnancy resulted from the third cycle. She underwent follow-up with regular pelvic ultrasonography. She was found to have a 10 cm complex left ovarian mass at 19 weeks' gestation. She underwent exploratory laparotomy and a left salpingo-oophorectomy. Histopathologic examination revealed a serous adenocarcinoma of low malignant potential. She experienced no problems after operation, and the pregnancy progressed to term. She had a cesarean section and complete surgical staging.

Case 3. A 28-year-old woman with primary infertility who underwent induction of ovulation with Pergonal, resulting in an intrauterine pregnancy after two cycles of therapy, was found to have a right adnexal mass and underwent follow-up with serial sonograms. She presented with an acute abdomen at 21 weeks' gestation, presumably from torsion of an ovarian cyst. She underwent exploratory laparotomy and ovarian cystectomy. The final pathologic examination revealed a serous adenocarcinoma of low malignant potential. After delivery of a viable infant, she had elective surgical staging.

Comment

The incidence of ovarian tumors of low malignant potential may not actually be increasing, but the diagnosis is being made earlier in this select group of patients who are under intense medical surveillance. They have frequent sonograms and undergo laparoscopy and a high percentage have cesarean section. It may be coincidental that ovarian carcinoma developed in this subgroup after induction of ovulation. If these cases had been undetected or undiagnosed, the women may have presented with late-stage ovarian carcinoma

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10 or 15 years later. We may have interrupted the natural history of the disease with earlier intervention and diagnosis.

These case reports are clinical and not scientific data. However, the clinical observation is important and one that we believe requires evaluation and follow-up. It might be prudent to set up a national registry for patients <35 years old with ovarian carcinoma. If this confirms our observation and the common denominator is infertility with subsequent ovulation induction, further study regarding this issue appears warranted. This may have an effect not only on the management of patients with infertility but also in the earlier diagnosis of patients with ovarian carcinoma.

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Maternal and fetal cardiovascular responses to strenuous bicycle exercise

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In a longitudinal study we investigated some cardiovascular responses to strenuous bicycle exercise in 33 healthy women during pregnancy and the postpartum period. The exercise electrocardiogram demonstrated depression of the ST segment in 12% of women in the absence of clinical signs of ischemia, and the incidence of these changes was unaffected by pregnancy. In spite of slightly different blood pressures at rest during the first and second trimesters of pregnancy, the blood pressure response to exercise at approximately 75% $\dot{V}O_2$max was virtually unaffected by pregnancy. After a maximal bicycle test, the fetal heart rate was increased by an average of 4 beats/min, without a change in pattern. Tocodynamometry suggested a transient increase in uterine activity after maximal exercise in 6% of the tests. These findings support the view that strenuous exercise of limited duration is not harmful to the healthy mother and fetus. (Am J Obstet Gynecol 1992;166:854-9.)

Key words: Pregnancy, maximal exercise, blood pressure, electrocardiogram, fetal heart rate

With ever-increasing numbers of pregnant women wanting to participate in sport activities, the question as to how safe maternal exercise is for mother and fetus becomes more important. With regard to the mother, cardiovascular adjustments to strenuous physical activities could be dangerous because of the added burden to an already hyperdynamic maternal circulation. On the other hand, the low resistance of the dilated vascular bed in pregnancy could have a protective effect on the circulation and depress the maternal blood pressure response to exercise.

Physical exercise of the mother presents a potential threat to fetal health because uterine blood flow is known to decrease progressively with exercise intensity.[1] Results of early studies in healthy pregnant women suggested that even mild to moderate exercise may occasionally be associated with fetal bradycardia during exercise,[2, 3] but this finding was later dismissed as representing an artifact.[4] Animal studies have demonstrated that several compensatory mechanisms act

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together to preserve fetal oxygen consumption even during exhaustive exercise.[1] These observations provide the justification for a study of maternal and fetal responses to strenuous exercise in healthy pregnant women.

In this study in healthy pregnant women with uncomplicated pregnancy, we assessed some physiologic responses to strenuous bicycle exercise relevant to the safety of the mother and her fetus: maternal electrocardiogram (ECG), blood pressure, uterine contractility, and fetal heart rate (FHR).

Material and methods

We studied 33 women at 16, 25, and 35 weeks' gestation and 7 weeks after delivery. In each study period we measured maternal blood pressure and recorded the ECG at rest and at increasing levels of bicycle exercise until maximal aerobic power was reached; we also recorded FHR and uterine contractility at rest and during recovery. All women were healthy and had uncomplicated singleton pregnancies. The study was approved by the Hospital and University Ethics Committee, and all women included in the study gave their informed consent.

Exercise was performed in an air-conditioned room at 21° C and 55% humidity. All participants underwent a physical and obstetric examination before each test to exclude any abnormality. In addition, we recorded FHR by means of Doppler ultrasound and uterine contractility by external tocodynamometry (model III fetal monitor, Corometrics, Wallingford, Conn.) in the second and third trimesters of pregnancy to ensure fetal well-being.

After 20 minutes of rest in the semisupine position, each woman was asked to sit on a bicycle ergometer (model II FE ergometer 400 L, Mijnhardt, Bunik, The Netherlands). She was connected to a gas flow meter (Oxycon 4, Mijnhardt) for a study of maximal oxygen consumption on which we reported separately.[5] The volunteer was also connected to an ECG monitor (model RM 102, Honeywell, Best, The Netherlands) and a bipolar ECG, lead CM_5, from manubrium to the left precordial position (V5), was continuously recorded (with a Cardiostat, Siemens, Stockholm). We measured blood pressure at 3-minute intervals with a standard sphygmomanometer (Erka-sphygmomanometer, Bad Tolz, Germany) and auscultation using Korotkoff sounds I and V to indicate systolic and diastolic blood pressures, respectively. After 5 minutes of baseline measurements at rest on the ergometer, the woman started to exercise. Three minutes of warming up at 30 W was followed by stepwise increments in exercise intensity of 10 W every 30 seconds until maximal oxygen consumption ($\dot{V}O_2max$) was reached. This was followed by 5 minutes of cooling down at 10 W. The cardiotocogram could not be recorded reliably during exercise, so it was recorded during 20 minutes of recovery with the woman in a semisupine position.

The ECGs were evaluated according to previously established criteria.[6] Mean blood pressures were calculated as $\frac{1}{3} \times$ (Systolic pressure + 2 × Diastolic pressure). The cardiotocograms were evaluated in a blinded fashion with the use of the Fisher score.[7] In addition, we calculated the average basal FHR during the last 5 minutes of rest and the first 5 minutes of recovery with the woman in a semisupine position and measured the frequency of contractions.

For each test period and each variable under consideration we computed mean values and standard errors of the mean. Analysis of variance and Student t tests were used to assess differences between paired variables. A p value of <0.05 was taken as the level of significance.

Results

The 33 women who participated in the study, 23 nulliparous and 10 parous, remained healthy, with uncomplicated pregnancies, throughout the study period, and they were delivered of healthy infants. Maternal age at the time of delivery was 30.9 ± 0.7 years (mean ± SE), gestational age was 40.3 ± 0.2 weeks, and birth weight was 3.43 ± 0.08 kg. Birth weight for gestational age, corrected for parity and fetal sex, was between the 10th and 90th percentiles of the reference curve in 28, <10th percentile in four, and >90th percentile in one infant.

All subjects were studied at 16.1 ± 1.0, 25.3 ± 0.7, and 35.0 ± 0.6 weeks of pregnancy and at 6.7 ± 1.4 weeks after delivery. Mean body weight at 16 weeks' gestation was 68.0 ± 1.7 kg, not different from the postpartum control value of 67.6 ± 1.9 kg, but it increased significantly with advancing gestational age to 71.8 ± 1.8 and 75.3 ± 1.8 kg at 25 and 35 weeks' gestation, respectively.

The values of heart rate at rest and at maximal exercise have been previously reported.[5] Heart rate at rest increased significantly with advancing gestational age from 87 ± 2 beats · min^{-1} at 16 weeks to 89 ± 2 beats · min^{-1} at 25 weeks and to 94 ± 2 beats · min^{-1} at 35 weeks' gestation, as compared with 83 ± 2 beats · min^{-1} post partum. Heart rate showed a linear increase with exercise intensity to a maximum of 174 ± 2 beats · min^{-1} throughout pregnancy and 178 ± 2 beats · min^{-1} post partum, as shown in Fig. 1. Analysis of the resting ECGs for abnormal changes in conduction, repolarization, and rhythm showed no anomalies in 30 women; two demonstrated sinus arrhythmia and one had an ectopic atrial rhythm. During exercise, 28 women had a normal ECG throughout pregnancy and post partum; four subjects showed

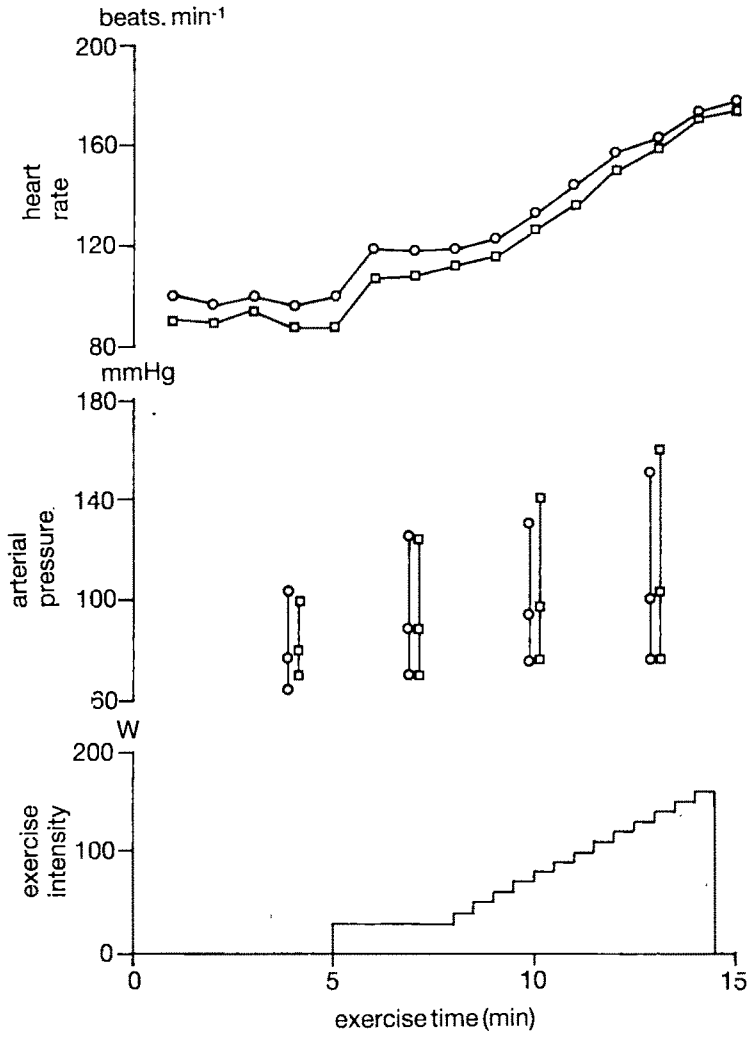


Fig. 1. Rise in heart rate and systolic and mean blood pressures at increasing levels of exercise intensity and absence of change in diastolic blood pressure. ○, First trimester; □, post partum.

depression of the ST segment of ≥0.1 mV, accompanied by an inverted T wave in one subject. Sinus arrhythmia disappeared during exercise in the two women who showed this abnormality at rest, but the ectopic atrial rhythm persisted in one subject. The ECG abnormalities during exercise in these women occurred consistently in all pregnancy and postpartum test periods. None of the 33 women had signs or symptoms suggestive of cardiac ischemia.

Mean values of systolic, diastolic, and mean arterial pressures at rest and at approximately 75% $\dot{V}O_2max$ are shown in Table I. Systolic blood pressures at rest appeared to be unaffected by pregnancy, whereas diastolic and mean pressures were slightly lower during the first and second trimesters of pregnancy as compared with values obtained in the third trimester and post partum. Systolic and mean arterial pressures showed a linear increase with exercise intensity (Fig. 1). At approximately 75% $\dot{V}C_2max$ systolic and mean arterial pressures were significantly increased by an average of 36% and 17%, respectively, whereas diastolic pressures increased marginally by an average of 2%. Mean values of exercise intensity and systolic blood pressure were similar and values of diastolic pressure were slighly lower in the first and second trimesters than in the postpartum period. During the third trimester the average exercise intensity at the time of the measurement was 5% higher and the systolic blood pressure during exercise was 4% higher than in the postpartum period, whereas the diastolic pressure was similar. Except for a marginal difference at 16 weeks' gestation, calculated mean blood pressures during ex-

Table I. Effect of pregnancy on blood pressure at rest and during strenuous bicycle exercise

	Blood pressure (mm Hg)			
	Systolic	*Mean*	*Diastolic*	*Exercise intensity (% $\dot{V}O_2max$)*
At rest				
16 wk	110.0 ± 1.8	85.4 ± 1.3*	73.0 ± 1.2*	—
25 wk	113.0 ± 1.7	87.5 ± 1.1*	74.7 ± 1.0*	—
35 wk	114.8 ± 1.9	90.3 ± 1.3	78.0 ± 1.3	—
Post partum	112.9 ± 1.6	90.6 ± 1.2	79.4 ± 1.1	—
During exercise				
16 wk	150.3 ± 2.7	100.8 ± 1.4†	76.1 ± 1.1*	74.0 ± 1.9
25 wk	153.9 ± 3.0	102.9 ± 1.5	77.4 ± 1.1†	74.5 ± 1.9
35 wk	158.0 ± 3.0*	105.6 ± 1.5	79.4 ± 1.2	76.8 ± 2.0
Post partum	151.5 ± 2.7	103.5 ± 1.3	79.5 ± 1.0	73.1 ± 2.1

Values are mean ± SE. Exercise values are significantly increased above resting values ($p < 0.01$) for all variables except for diastolic blood pressure.

*$p < 0.01$, compared with postpartum control values ($n = 33$).

†$p < 0.05$, compared with postpartum control values ($n = 33$).

ercise in pregnancy were not different from the postpartum value.

Mean FHR during the first 5 minutes after exercise showed a small but significant increase relative to the resting control value observed in the second and third trimesters; in the second trimester it rose from 143.9 ± 0.9 to 148.7 ± 1.5 beats · min^{-1} and in the third trimester from 140.7 ± 1.4 to 144.2 ± 1.8 beats · min^{-1}. The Fisher score could not be established in 10 of the 33 subjects at 25 weeks' gestation because of poor quality of tracings, whereas all 20-minute tracings except one were satisfactory at 35 weeks' gestation. Average scores after exercise were not significantly different from those obtained at rest, both at 25 weeks' gestation (9.7 ± 0.2 and 9.9 ± 0.1, respectively) and at 35 weeks of pregnancy (9.5 ± 0.2 and 9.6 ± 0.2, respectively). At 25 weeks of pregnancy 22 of the remaining 23 fetuses had an optimal score of 8 to 10 points both at rest and after exercise. In one case the score changed from optimal (8 points) at rest to suboptimal (7 points) after exercise. At 35 weeks 30 fetuses were scored as optimal both before and after exercise, one changed from optimal (8 points) to suboptimal (7 points), and one changed from suboptimal (7 points) to optimal (10 points).

Uterine activity was recorded reliably from all subjects in both trimesters. At 25 weeks' gestation 32 of the 33 women showed no uterine activity before or after exercise; in one woman uterine contractility after exercise was not present at rest but was recorded after exercise. At 35 weeks' gestation 27 women had no uterine contractility either before or after the bicycle exercise, three women showed some uterine contractility both before and after exercise, and three women showed a few irregular contractions after exercise, which subsided spontaneously. None of the women noticed the recorded contractions or progressed into labor.

Comment

We observed depression of the ST segment in 12% of the pregnant women studied; similar[8] or much higher figures of up to 75%[9] have been observed in other studies in healthy pregnant women. The cause of these repolarization abnormalities remains unknown; most women with ST depression during exercise testing have angiographically normal coronary arteries.[10] It has been suggested that the ECG changes could be due to altered sympathetic regulation,[10] and one might speculate the ECG abnormalities during exercise may occur more frequently in pregnant women[11] because pregnancy may affect sympathetic regulation.[12] Our findings do not suggest that pregnancy markedly increases the incidence of repolarization abnormalities or arrhythmias in exercising women, but our study does not rule out a possible effect of pregnancy. Our data seem to support, however, the suggestion that exercise electrocardiography is of limited value in healthy pregnant or nonpregnant women.[13]

We used sphygmomanometry and auscultation for the measurement of arterial blood pressure. This method has known limitations with respect to accuracy and precision, in the nonpregnant and pregnant state.[14] However, in this longitudinal study the errors inherent in the method of blood pressure measurement may be expected to have been similar in all test periods, and for that reason it seems unlikely that they have affected pregnancy trends. In the first and second trimesters of pregnancy we found lower diastolic pressures at rest as compared with those observed in the third trimester

and post partum but no significant differences in systolic blood pressure. This is in agreement with the literature and is thought to reflect a reduction in systemic vascular resistance.[14] Systolic arterial pressure increases with exercise intensity, whereas diastolic pressure increases only slightly.[15] Some authors reported a higher systolic pressure response to exercise in pregnant women than in nonpregnant ones,[16] whereas others observed no difference.[17, 18] We found that absolute values of diastolic and mean arterial pressure during exercise were slightly lower during the first and second trimesters of pregnancy, which may reflect the lower resting values observed during these periods. Systolic blood pressure during exercise differed from that in the postpartum period only in the third trimester of pregnancy, when it was significantly increased by 4%, or 6 mm Hg. This may reflect the cumulative effect of the 2% higher systolic pressure at rest and the 5% higher exercise intensity during the third trimester in this study rather than a physiologically important difference in response. The observation that the blood pressure response to exercise is virtually unaffected by pregnancy in spite of the hyperdynamic circulation suggests that the pressure response is governed by local demand.

The FHR response to moderately strenuous maternal exercise has been investigated repeatedly since it was proposed as a clinical test for uteroplacental insufficiency.[19] We consistently observed an elevated basal FHR after the maximal bicycle test. This probably reflects the increase in body temperature rather than fetal distress and confirms similar observations after less strenuous exercise.[20, 21] Although abnormal heart rate patterns, including bradycardia, have been reported during exercise,[2, 3] other studies have adduced evidence that these abnormal patterns are artifacts caused by rhythmic maternal movements during exercise.[4] During recovery from exercise such artifacts are less likely to occur. Fetal bradycardia has been reported to persist occasionally for 4 minutes after exercise,[2] and one study reported a 19% incidence of fetal bradycardia (defined as a heart rate <110 beats/min for ≥ 10 seconds) within a few minutes after maximal maternal exercise before return to a normal heart rate.[22] One may only speculate as to what may have caused these changes. We were unable to record FHR reliably during the bicycle test but found the pattern unchanged after exercise and 5 minutes of cooling down. We did not measure FHR within 5 minutes of maximal effort. However, with the use of invasive techniques it has been demonstrated that even exercise at the point of exhaustion has little effect on FHR in experimental animals and that fetal blood gas values remain within normal limits.[23]

We observed uterine contractility after exercise in some women. This is in agreement with observations by others who reported either occasional slight increases[24] or no change[25] in uterine contractility in response to physical activity, without progression into labor.

In conclusion, we obtained no evidence that strenuous exercise of limited duration in pregnancy markedly modulates the ECG or blood pressure response or affects the FHR pattern. The safety to the healthy mother and fetus of strenuous exercise of long duration remains to be studied.

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Is trichomoniasis often associated with bacterial vaginosis in pregnant adolescents?

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The same criteria for identifying bacterial vaginosis are often present in women with trichomoniasis. These criteria include elevated vaginal pH, vaginal odor, homogeneous discharge, increased anaerobic bacteriologic vaginal flora, and elevated levels of bacterial enzymes. Clinically mixed vaginal infections occur, and because the treatment for these two conditions can be different, it is important to distinguish between them. Trichomoniasis can interfere with a Gram stain diagnosis or the proline aminopeptidase test for bacterial vaginosis. Clue cells are not generally found in women with *Trichomonas vaginalis*, but when present, they strongly indicate the concomitant presence of bacterial vaginosis. (AM J OBSTET GYNECOL 1992;166:859-63.)

Key words: Bacterial vaginosis, trichomoniasis, clue cells

Older studies report an association between trichomoniasis and bacterial vaginosis, with as many as 80% of women infected with *Trichomonas vaginalis* also being diagnosed with bacterial vaginosis.[1-3] For the diagnosis of bacterial vaginosis, Amsel's classic criteria and, more recently, other more accurate criteria have been identified to objectively and rapidly make a correct diagnosis.[4-6]

Bacterial vaginosis is a condition in which there is a grossly abnormal vaginal ecosystem with a predominance of anaerobic flora and decreased lactobacillus species, creating an environment conducive to anaerobic proliferation. *T. vaginalis* is an anaerobic protozoan capable of creating and thriving in an anaerobic milieu. Trichomonads contain granules called hydrogenosomes, which generate hydrogen. Hydrogen combines with oxygen to form water, thereby removing oxygen from the vaginal ecosystem.[7] This mechanism stimu-

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lates and facilitates the growth of other anaerobic bacteria.[8]

Mixed vaginal infections are seen frequently in clinical practice, especially in sexually transmitted disease clinics.[2] It is unclear, when two or more types of vaginitis occur in an individual, whether criteria used to diagnose one type of vaginitis are affected by the presence of another pathogen (i.e., bacterial vaginosis in the presence of trichomoniasis). It has been established that if women do not undergo culturing for *T. vaginalis*, the disease will be underdiagnosed, making study analysis of patient groups difficult. The purpose of this study was to clarify the interrelationship between trichomoniasis and bacterial vaginosis. Trichomonads cause some manifestations seen in bacterial vaginosis, but conceptually there are two distinct entities that should be able to be differentiated. Six different diagnostic methods are currently in use to diagnose bacterial vaginosis. All methods were studied to see how the presence of trichomoniasis affects each of these various criteria.

Material and methods

After informed consent was obtained, 142 consecutive pregnant teenagers were tested for both bacterial vaginosis and trichomoniasis and for sexually transmitted diseases at their initial visit to the Adolescent Pregnancy Program at Sinai Samaritan Medical Center between March 1989 and March 1990. All but 33 were primigravid with a mean age of 15.9 ± 1.82 years. The mean gestation when they were first seen was 17.4 ± 8.8 weeks. Median gravidity was 1, parity 0, abortion 0.

Trichomonads were detected by two methods: analysis of saline solution wet mounts of vaginal secretion and by culture of vaginal secretions in Diamond's medium modified (Remel, Lenexa, Kan.). Wet mounts were prepared by placing a saline solution–moistened, cotton-tipped swab containing vaginal secretions, obtained from the lateral walls of the anterior vaginal fornices, into 1.0 ml of physiologic saline solution. A drop of this suspension was placed on a slide and a coverslip was added. Examination was made at a magnification of ×100 with >20 fields being examined. Any suspicious elements were examined at a magnification of ×400. Only motile trichomonads were counted. Wet mounts were prepared within 15 minutes of collection and analyzed immediately. The swab was then placed into trichomonas culture medium and incubated at 35° ± 2° C under 5% to 7% carbon dioxide at 80% humidity. The medium was examined at 2, 4, and 7 days for motile organisms.

Bacterial vaginosis was detected by six methods. Two clinical methods (Amsel et al.[3] and Thomason et al.[6]), three Gram stain interpretative methods (Spiegel et al.,[4] Nugent et al.,[5] and a new rapid semiquantitative method), and a proline aminopeptidase assay[9] were used. Vaginal secretions were collected as previously described, and wet mounts were examined at a magnification of ×400 for clue cells.[6] Odor was tested by immersing a swab containing freshly collected vaginal secretions into 1.0 ml of 10% potassium hydroxide and then smelling the swab. Only a distinctly fishlike odor was considered positive. The pH of vaginal secretions was measured with phenaphthazine paper (Nitrazine paper, E.R. Squibb and Sons, Princeton, N.J.). The presence of homogeneous discharge was noted during routine pelvic examination. The criteria of Amsel et al. for bacterial vaginosis include three of four diagnostic signs: presence of homogeneous discharge, pH >4.5, positive amine odor, and presence of clue cells on wet preparation.[2] Criteria of Thomason et al. include the presence of clue cells on microscopic analysis of vaginal secretions.[6]

Gram stains were made from smears of vaginal secretions. For the Spiegel and Nugent method at a magnification of ×1000, the following coding scheme was used: 1+, less than one organism per field; 2+, one to five organisms per field; 3+, six to 30 organisms per field; 4+, >30 organisms per field. Patients were considered to have bacterial vaginosis by the Spiegel method of interpretation if lactobacillus morphotypes were <2+, *Gardnerella* morphotypes were 4+, and at least one other morphotype was present.[4] Patients were considered to have bacterial vaginosis by the Nugent Gram stain method if the following criteria were met: Numeric values were assigned to each quantitation of morphotype, specifically, 4+ lactobacilli = 0, 3+ lactobacilli = 1, 2+ lactobacilli = 2, 1+ lactobacilli = 3, no lactobacilli = 4, 1+ *Gardnerella* = 1, 2+ *Gardnerella* = 2, 3+ *Gardnerella* = 3, 4+ *Gardnerella* = 4, 1 to 2+ *Mobiluncus* = 1, 3 to 4+ *Mobiluncus* = 2. If the number achieved from the counts of these three morphotypes was 7 to 10, the patient was diagnosed as having bacterial vaginosis. If the counts gave a numeric value of 4 to 6, the patient's flora was considered intermediate, and if the counts gave a numeric value of 0 to 3, bacterial vaginosis was excluded.[5] In addition, all Gram stains were examined for the presence of clue cells. A semiquantitative Gram stain was also used. If clue cells were present and nonlactobacillus morphotypes exceeded lactobacillus morphotypes, bacterial vaginosis was diagnosed.

The proline aminopeptidase assay was performed as originally described.[9] Briefly, a cotton-tipped swab containing vaginal secretions was placed into 1.2 ml saline solution and agitated to suspend the vaginal material. The swab was removed and the suspension was centrifuged at 8730*g* for 2.5 minutes on a Microfuge (Beckman, Palo Alto, Calif.) to pellet the suspension. All but 0.1 ml of the supernatant was discarded. The pellet was then resuspended and 0.05 ml was added to a microtiter

Table I. Vaginal discharge characteristics of pregnant adolescents

Characteristic	Study group (n = 120)			
	T. vaginalis present (n = 32)		*T. vaginalis absent* (n = 88)*	
	No.	*%*	*No.*	*%*
Clinical criteria				
Odor with 10% potassium hydroxide	5	16	27	31
Homogeneous discharge	27	84	61	69
pH >4.5	19	59	43	49
Clue cells in wet mount	2	6	29	33
Nonlactobacilli morphotypes predominate	24	75	64	73
Increased white blood cells	17	53	35	30
Bacterial vaginosis by Amsel et al.	7	22	29	33
Gram stain criteria				
Bacterial vaginosis by Spiegel et al.	10	31	29	33
Bacterial vaginosis by Nugent et al.	12	38	39	49
Bacterial vaginosis by semiquantitative method	2	6	39	49
Proline aminopeptidase	20	62	29	33

*Includes patients with yeast or bacterial vaginosis by criteria of Amsel et al.

well containing 0.05 ml of 0.2% L-proline β-naphthylamide (Sigma, St. Louis). The trays were covered to prevent evaporation and incubated at 35° ± 2° C for 4 hours. Then 0.05 ml of 0.015% fast garnet GBC salt (Sigma) was added to the well to detect β-naphthylamine and read visually for red color after 5 minutes.

χ^2 Statistical methods were used to analyze data. Both McNemar and Fisher tests were used where appropriate.

Results

Of the 142 teens surveyed, 22 (15%) were excluded because of having simultaneous chlamydia or gonorrhea. Women with vaginal yeast infections were not excluded because it has been shown that yeasts do not affect the diagnosis of either trichomoniasis or bacterial vaginosis.[9,10] Thirty-two of 120 evaluable teens (26.6%) were found to have trichomonads by culture. Only 25 cases of trichomoniasis, 78% of those diagnosed by culture, were also diagnosed by wet mount examination ($p < 0.05$, McNemar χ^2). No teens with positive wet mounts for trichomonads had negative cultures.

Table I shows the association between various diagnostic criteria used to identify women with bacterial vaginosis and trichomoniasis in the 120 evaluable teens. Of the 88 patients who had no trichomonads (Table I, column 2), a subset of 59 teens had no trichomonads by culture and no bacterial vaginosis (defined by criteria of Amsel et al.). This group's characteristics are shown in Table II.

Table II. Vaginal discharge characteristics in pregnant adolescents ($n = 59$) who were culture negative for trichomonads and did not have bacterial vaginosis*

Characteristic	Patients	
	No.	*%*
Clinical criteria		
Odor with 10% potassium hydroxide	0	—
Homogeneous discharge	40	68
pH >4.5	19	32
Clue cells in wet mount	0	—
Nonlactobacilli morphotypes predominate	28	47
Increased white blood cells	20	34
Gram stain criteria		
Bacterial vaginosis by Spiegel et al.	6	10
Bacterial vaginosis by Nugent et al.	3	5
Bacterial vaginosis by semiquantitative method	4	7
Proline aminopeptidase	4	7

*Bacterial vaginosis diagnosed by criteria of Amsel et al.

Teens with trichomonads (Table I) were less likely to have odor on alkalinization of vaginal secretions than teens without trichomonads ($p < 0.05$, Fisher) and slightly more likely to have homogeneous discharge, although this finding was not significant ($p > 0.05$, Fisher). Clue cells in wet mount examination were found in only two teens with trichomonads, both of whom had bacterial vaginosis by all diagnostic methods used.

There was evidence of change in the bacterial flora, with teens having trichomonads significantly more likely to have nonlactobacilli morphotypes predominating when compared with the group of teens in Table II ($p < 0.05$, Fisher). Twenty teens with trichomonads had positive proline aminopeptidase results, but only seven had bacterial vaginosis by any criteria used. Women with trichomonads consistently had leukocytes exceeding vaginal epithelial cells when compared with the teens in Table II (53% vs 34%, $p < 0.05$, Fisher).

Only seven teens having trichomonads were simultaneously diagnosed with bacterial vaginosis by criteria of Amsel et al. (Table I). All had positive results by proline aminopeptidase. Of significant interest is the fact that only two (2/32) teens with trichomoniasis had clue cells. All women with trichomonal vaginitis who had clue cells in secretions also had concomitant bacterial vaginosis by any of the six methods used to diagnose bacterial vaginosis.

From the total 120 evaluable teens, 36 women were diagnosed as having bacterial vaginosis with the criteria

Table III. Comparison of diagnostic criteria for bacterial vaginosis in pregnant teens ($n = 120$)

	Bacterial vaginosis present	
	No.	*%*
Clinical criteria		
Amsel et al.	36	30
Thomason et al.	31	26
Gram stain criteria		
Spiegel et al.	39	32
Nugent et al.	34	28*
Semiquantitative method	36	30
Proline aminopeptidase	32	27

*Eleven women also had intermediate flora.

of Amsel et al. and 31 of 36 would have met the criteria of bacterial vaginosis by Thomason et al. (clue cells in wet mounts) (Table III). There were 13 (13/36) women with bacterial vaginosis who had all of the four diagnostic characteristics of Amsel et al. All women in this study who had clue cells on wet mount also had clue cells on Gram stain, but six additional patients had clue cells on Gram stain yet did not meet any criteria for bacterial vaginosis nor have trichomonads by culture.

Thirty-nine women had bacterial vaginosis by Spiegel's Gram stain, including 30 of 31 who had clue cells on wet mount (Table III). Nugent's Gram stain method showed 34 teens with bacterial vaginosis but also showed 11 with intermediate classification, eight of whom had trichomonads. There were 88 teens who had nonlactobacilli morphotypes exceeding lactobacilli types, but if the two criteria, clue cells on Gram stain and nonlactobacilli morphotypes exceeding lactobacilli on Gram stain, were combined, 36 women were identified, 31 of whom had bacteria vaginosis by criteria of both Amsel et al. and Thomason et al.

Forty-nine (49/120, 41%) women had positive test results by the proline aminopeptidase assay. Sixty-two percent (20/32) of women having trichomonads were also found to have a positive proline aminopeptidase assay, although only seven of this group had concomitant bacterial vaginosis by criteria of Amsel et al. (Table I). One teen with a positive result by proline aminopeptidase had neither trichomonads nor bacterial vaginosis by any of the other methods. Proline aminopeptidase accurately identified 89% (32/36) of women having bacterial vaginosis by the criteria of Amsel et al. in this study and 97% (30/31) having bacterial vaginosis by criteria of Thomason et al. (Table III).

Comment

The association of trichomoniasis and bacterial vaginosis (7/32, 22%) diagnosed with classic criteria in asymptomatic pregnant teens was not as great as previously reported in symptomatic women[1] or in women attending sexually transmitted disease clinics.[3] Study patients were all pregnant, and the effect pregnancy has on the association between trichomoniasis and bacterial vaginosis is not known. Nearly half of the women with trichomoniasis lacked increased levels of white blood cells in vaginal secretions. However, if trichomonads were detectable on wet mount, white blood cells were elevated in 68% (17/28) of cases. Women with trichomoniasis nearly always had abnormal bacterial flora, i.e., lactobacilli morphotypes were outnumbered by nonlactobacilli morphotypes. Of significance is the finding of a lack of clue cells in teens having *T. vaginalis* in secretions. On the contrary, if clue cells were present in teens with trichomoniasis, all had bacterial vaginosis. Certainly, if an observer finds trichomonads and clue cells during microscopic examination of vaginal secretions, other criteria for bacterial vaginosis should be sought.

Homogeneous discharge and vaginal pH continue to be diagnostic signs that lack specificity, as has been suggested in previous studies.[6, 10, 11] Our data in Table II confirm this finding. Observer bias and pH differences in sample sites may account for these factors being present in a large number of women without *T. vaginalis* or bacterial vaginosis. Many women during pregnancy complain of an increased amount of vaginal discharge, and this may simply reflect hormonal changes with increased vascularity present in the lower genital tract during this time period. Perhaps this explains why 68% of our population were found to have homogeneous discharge.

Bacterial vaginosis was found in 30% (36/120) of our teens, yet the predominance of nonlactomorphotypes (73%, 88/120) was seen even more frequently. This criterion is easily identified on Gram stain and wet preparation analysis of vaginal secretions. However, this study confirms previous work that using this single criterion on Gram stain or Papanicolaou smear to accurately predict bacterial vaginosis would result in overcalling the diagnosis of bacterial vaginosis.[6] In Table II this single criterion would have falsely identified nearly half of these adolescents as having bacterial vaginosis.

Proline aminopeptidase is a rapid, objective biochemical test that has a positive result in women having bacterial vaginosis.[9] The data in this study show that this assay is affected by trichomonads, presumably because of the changes in bacterial flora induced by *T. vaginalis* when it removes the oxygen from the vaginal environment. Vander Meijden et al.[12] have shown that the microbial flora in bacterial vaginosis and trichomoniasis is similar. This may explain our finding of bacterial flora in trichomoniasis causing a positive proline test. In this

study 4-hour incubation with substrate was used for the proline aminopeptidase assay. Shortening the incubation time might alleviate the problem of false-positive results caused by trichomonads. However, clinicians should be aware of the high false-positive rate of this test seen in women having *T. vaginalis* alone and not bacterial vaginosis in vaginal secretions. Concomitant wet mount examination of vaginal secretions is always prudent before proline aminopeptidase is used as a screening tool for the identification of women having bacterial vaginosis.

In conclusion, in a population that had cultures for *T. vaginalis,* this protozoan was not frequently associated with bacterial vaginosis. Such women may have some of the characteristics that meet the criteria for bacterial vaginosis, such as profuse discharge with odor or elevated vaginal pH, but they usually have no clue cells. The absence of clue cells strongly suggests that these women do not have bacterial vaginosis.[6, 10, 11, 13] However, if women who have trichomonads in secretions also have clue cells, bacterial vaginosis should be strongly suspected. Bacterial vaginosis has been associated with both premature rupture of membranes and premature labor, and therapy in such patients should be considered.[11, 13] Single-dose metronidazole therapy, while effective for trichomoniasis, is not effective for bacterial vaginosis.[11] Similarly, because there is a change in bacterial flora induced by trichomonads, both the Spiegel et al. and Nugent et al. Gram stain interpretations used for diagnosis of bacterial vaginosis, and also the proline aminopeptidase assay can give false-positive diagnoses for bacterial vaginosis when trichomonads are present. In contrast, concomitant yeast infection does not influence bacterial flora and therefore affects neither clinical criteria for bacterial vaginosis nor the Gram stain diagnostic method nor the proline aminopeptidase assay.[9] Because trichomoniasis can cause false diagnoses of bacterial vaginosis, women treated with clindamycin for bacterial vaginosis who fail to respond should be tested for trichomoniasis and treated with metronidazole if trichomonads are found, because clindamycin has no effect on *T. vaginalis.*

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Laparoscopic radical hysterectomy with paraaortic and pelvic node dissection

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We report the first case of a laparoscopic radical hysterectomy and paraaortic and pelvic lymphadenectomy to treat a stage IA2 carcinoma of the cervix. To our knowledge, a laparoscopic radical hysterectomy with laparoscopic paraaortic lymphadenectomy has not been previously described. (AM J OBSTET GYNECOL 1992;166:864-5.)

Key words: Laparoscopy, paraaortic node dissection, radical hysterectomy

We report the first case of a laparoscopic radical hysterectomy and paraaortic and pelvic lymphadenectomy to treat a stage IA2 carcinoma of the cervix.

Case report

A 30-year-old woman, gravida 3, para 2-0-0-2, was evaluated at 12 weeks' gestation for an abnormal Papanicolaou smear. Colposcopic examination was satisfactory and findings were consistent with dysplasia. No areas suspicious for invasion were noted. Biopsy revealed grade 3 cervical intraepithelial neoplasia. She underwent follow-up during pregnancy with colposcopy and biopsy without evidence of invasion and was delivered by repeat cesarean section.

Laser conization (6 weeks post partum) revealed cervical intraepithelial neoplasia with a 2.74 mm focus of invasion with lymphatic space involvement and a width of 3 cm. Lymphatic space involvement by tumor cells put this patient at higher risk for metastases and consequently made her a candidate for radical hysterectomy. Physical examination revealed a healthy young woman, 5 feet 8 inches tall, weighing 199 pounds, with a history of three cesarean sections.

A standard bowel preparation was used and she received three 2 gm doses of cefoxitin prophylactically.

During the laparoscopic portion of the procedure all pedicles were coagulated with bipolar cautery and incised with the carbon dioxide laser.[1] Sutures were not required and peritoneal incisions were allowed to heal secondarily.

For the right common iliac and lower paraaortic lymphadenectomy, the patient was tilted to the left side and placed into the deep Trendelenburg position at 35 to 40 degrees. The ureter was identified through the peritoneum. An opening was made into the peritoneum just above the sacral promontory; with hydrodissection[2] the retroperitoneal space was injected with lactated Ringer's solution and then incised with the carbon dioxide laser toward the duodenal bulb.[2] The common iliac and paraaortic nodes were removed from the veins' surface by blunt dissection, hydrodissection, and the carbon dioxide laser.[1] Larger bleeders, such as the venous perforators of the vena cava, were coagulated first with bipolar cautery.[1] The ureter was identified immediately next to the vena cava, and dissection was taken to 2 cm above the aortic bifurcation. Then the rectovaginal, paravesical, and pararectal spaces were dissected. The rectum was dissected off the posterior vaginal wall. This was taken to 5 cm below the cervix. The round ligaments were coagulated close to the pelvic sidewalls and transected. The peritoneum was opened and the paravesical and pararectal spaces were dissected out, allowing excellent skeletonization of the obliterated hypogastric artery.[2] The uterine vessels were identified, skeletonized, coagulated medial to their origin, and transected, then rotated anterior to the ureters.[2]

The vesicovaginal space was developed using techniques described above, with difficulty because of scarring from three previous cesarean sections.[2]

The uterine vessel was disseted medially for 4 cm and elevated from the ureter using the carbon dioxide laser. The anterior parametrium was transected, and the ureters were freed from the peritoneum and skeletonized down to the bladder. The uteroovarian pedicles were coagulated and transected.[1]

The left common iliac nodes, with the left pelvic nodes, were removed as on the right side.

The external iliac nodes between the external iliac vein and artery and the obliterated hypogastric artery were carefully stripped to the deep circumflex veins. The obturator nerve was exposed and the hypogastric and obturator nodes were removed. The obturator nerve was dissected and cleaned caudally to the point

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at which it left the pelvis. The nodal tissue between the obturator nerve and external iliac vein was dissected. The inferior aspect of the external iliac vein was separated until the internal obturator muscle and pelvic bone were seen. Venous anastomosis between obturator and external iliac veins was visualized and retained. Lymph nodes posterior to the obturator nerve were excised. The uterosacral ligaments and lateral parametria were coagulated and sequentially transected approximately 1.5 cm lateral to the cervix. The dissection was taken down to 3 cm below the cervix. The vagina was entered anteriorly and posteriorly. Sponges in the vagina prevented loss of pneumoperitoneum.

The radical hysterectomy was completed vaginally by incising the vagina 3 cm distal to the cervix. After anterior and posterior mobilization, the residual cardinal ligaments were divided approximately 1.5 cm lateral to the cervix and then ligated with sutures.[1] The uterus and pelvic nodes were removed transvaginally.[1] The vaginal vault was then closed and a suprapubic catheter was placed.

The procedure lasted 7 hours with an estimated blood loss of 30 ml. The patient began eating within 24 hours and was discharged in 48 hours. The suprapubic catheter was removed in the office 7 days after operation.

The final pathologic examination revealed no residual cancer. All lymph nodes were negative. Of five paraaortic nodes removed, the largest was 1.5 cm and total tissue measured 4 × 3 × 3 cm. Nine left pelvic nodes were removed; the largest was 2.5 cm and total measurement was 6 × 5 × 2 cm. The five nodes removed from the right pelvic area totaled 5.5 × 2 cm; the largest was 1.3 cm. The measurement from the vaginal cuff's lower portion to the superior portion of the uterine fundus was 10.5 cm, and the uterine fundus was 5 × 5 cm wide. The hysterectomy specimen weighed 118 gm.

Comment

Before fair assessment of morbidity from operative laparoscopy can be made, more experience with similar cases is necessary. Only time will show if this case remains a surgical curiosity or whether the procedure becomes commonplace in the future.

Addendum

Since submitting this article, we have performed another laparoscopic radical hysterectomy with paraaortic and pelvic node dissection. The patient was 31 years old, gravida 0, with stage IA2 squamous cell carcinoma of the cervix. A total of 25 pelvic nodes (15 right, 10 left) and 9 paraaortic nodes were removed. Additional specimens included vaginal cuff measuring 3 cm in length and 3 cm of parametrium. The duration of the procedure was 6 hours. No intraoperative or postoperative complications were noted. She was discharged on the second postoperative day. Four months after operation the patient is doing well and shows no sign of recurrent malignancy.

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Emergency room use of transvaginal ultrasonography by obstetrics and gynecology residents

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OBJECTIVE: The objective of this study was to evaluate the feasibility and diagnostic value of the emergency room use of transvaginal ultrasonography operated by the obstetrics and gynecology residents.

STUDY DESIGN: One hundred sixty-eight patients scanned in the emergency room constituted the study group and were compared with 61 patients for whom emergency room scanning was not available. Scanning and no-scanning days alternated for a 6-month period. The Student *t* test was used in the analysis.

RESULTS: By comparing the two patient groups a statistically significant ($p < 0.0001$) difference was seen in the time spent by the group of patients scanned in the emergency room (40 minutes) when compared with that of the patients for whom no emergency room scanning was available (215 minutes). The residents expressed high confidence in the scanning procedure. There were six misdiagnosed cases, three of them nonruptured ectopic gestations. However, none of these patients was discharged without adequate treatment.

CONCLUSION: The study confirmed our hypothesis that obstetrics and gynecology residents can operate and should have available ultrasonography machines in the emergency departments to render quicker and better patient care. (AM J OBSTET GYNECOL 1992;166:866-72.)

Key words: Emergency room, transvaginal ultrasonography

For the past 10 years transabdominal ultrasonography has been actively used in the labor and delivery suites, has become a component part of resident education,[1] and has been successfully applied in emergency room care of women. Jehle et al.[2] reported on a study in which transabdominal ultrasonography was used by emergency room physicians to correctly diagnose, among nonobstetric problems, the presence or absence of intrauterine pregnancy in patients with lower abdominal complaints. The authors concluded that ultrasonography improved the diagnosis and treatment of a large number of patients and suggested that it should become a standard part of emergency room care. Later, Stabile et al.,[3] Grumbach et al.,[4] and Shea and Aghababian[5] also evaluated the frequency of abdominal and pelvic ultrasonography use in the emergency department. These authors found that the use of ultrasonography allowed for faster and safer discharge of patients. Furthermore, they found it to be cost-effective and helpful because many patients who normally would be admitted for observation, further laboratory testing, or operative techniques, could be discharged. Other publications confirmed the importance of ultrasonography in emergency rooms.[6-8]

Ultrasonography has been designated by the Council on Resident Education in Obstetrics and Gynecology as a component of resident education. Thus this laboratory instrument becomes fully integrated into acceptable patient care routines. However, the boundaries for its use should be established, and with this in mind we chose to look at the emergency room use of vaginal ultrasonography.

The American College of Emergency Physicians issued a position statement on the use of ultrasonography for emergency department patients.[9] They stressed the time dependency of the diagnosis in patients seen in the emergency room, i.e., life-threatening situations, urgent cases, and nonemergency cases in which ultrasonography is used as a procedural aid. Patients with a suspected ectopic pregnancy are part of the first two categories and therefore should be scanned in the emergency room.

Our hypothesis was that the availability of an ultrasonography machine in the majority of obstetrics and gynecology patients seen in the emergency room would enable the provider to spend less time in reaching the diagnosis and confirm the one previously made.

Residents in our department were being trained in the use of ultrasonography in general and transvaginal ultrasonography in particular. It was our belief that most residents mastered the technique in a short time,

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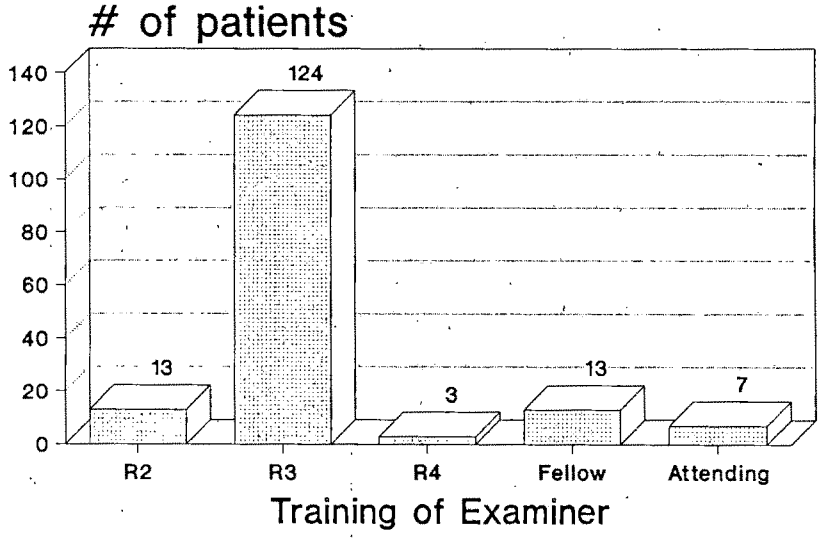


Fig. 1. Who performed the scan (n = 160)?

and they were able to perform transabdominal and transvaginal ultrasonographic examinations at a level that allowed them to incorporate the device into their routine in the emergency room history, physical examination, and laboratory studies. Thus the study was based on evaluating resident performance.

Material and methods

A Hewlett Packard (HP Co., Melville, N.Y.) Research and Development scanner with both a 3.5 MHz mechanical abdominal probe and a 6.5 MHz vaginal probe were used. The images were recorded on a Sony P-61 thermal video printer. The study period was 6 months, from April to September 1990. Two groups were created by alternating scanning days with no-scanning days. On the scanning days the patients were examined in the usual fashion by the residents. If an abdominal or transvaginal ultrasonographic examination was needed, this was performed by that resident. A report was then generated and a study questionnaire was completed. Consultation was available, if requested, during working hours.

On the no-scanning days patients were managed in the usual fashion customary before the ultrasonography unit was placed in the emergency room. If there was no emergency, an appointment for a later scan was given by the resident establishing a working diagnosis. If the patient required an ultrasonography examination, she was taken by that resident to the ultrasonography unit situated on the twelfth floor of the same building. After hours, the patient was either scanned when a consultation was available, admitted for observation if necessary, or discharged for ambulatory follow-up if the level of concern was such that delay in confirming the diagnosis would not place the patient at risk. The time spent by the patient in the emergency room and the time of admission or discharge after a final diagnosis was given were also recorded. The patients were followed up to establish the validity of the final diagnosis and the treatment, as well as the time of the final treatment received by the patient. The questionnaire had the following 14 components:

1. Length of time until final clinical diagnosis was achieved
2. Was it a scanning or no-scanning day?
3. Diagnosis before ultrasonography if on scanning day
4. Was the diagnosis changed after ultrasonography?
5. Did ultrasonography confirm the working diagnosis?
6. Was the management changed after ultrasonography?
7. Was an ultrasonography consultation obtained to clarify the situation?
8. Who performed the ultrasonographic examination?
9. Time of day that examination was done
10. Length of time in minutes to perform the scan
11. Was the scan abdominal, vaginal, or both?
12. The resident's opinion about whether it helped the diagnosis
13. The resident's evaluation of whether it was helpful for the provider
14. The level of confidence of the provider administering the ultrasonographic examination

On the scanning days, 168 patients were scanned and 61 patients constituted the control group seen in the emergency room on the no-scanning days. Most of the time all questions were answered by the scanning person; however, in a minority of the cases one or another question was answered in a fashion that could not be

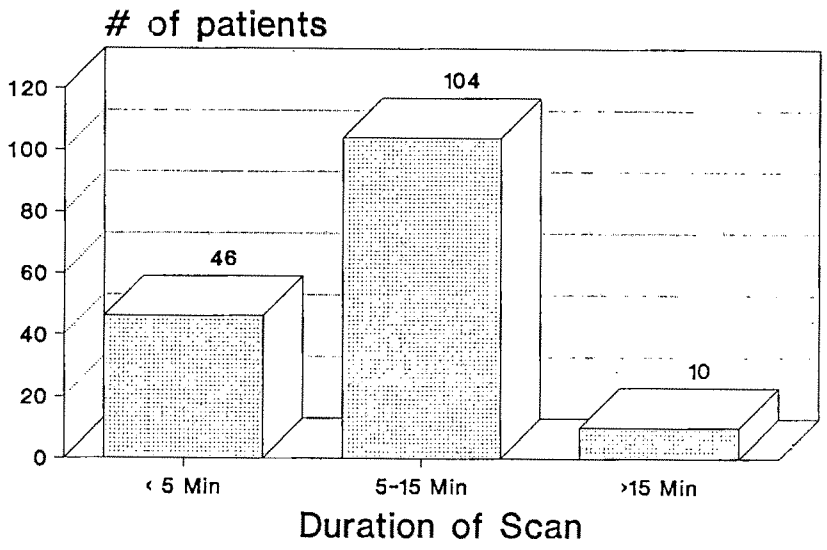


Fig. 2. Duration of scans (n = 160).

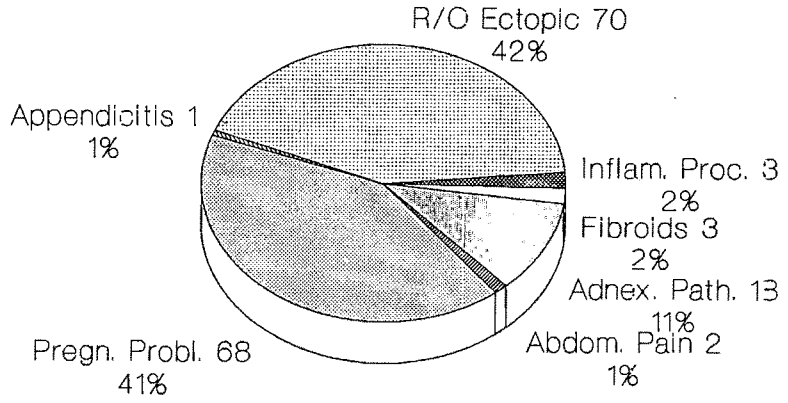


Fig. 3. Diagnosis before scans (on scanning days) (n = 167).

evaluated by us. Thus some graphs have slightly different patient counts. The discrepancy between the study and control group stems from the fact that 3 months after the beginning of the study the residents found ways to scan patients on the no-scanning days. Therefore we used the 61 controls that were handled according to the basic requirements of the protocol so that the study and control groups remained statistically comparable.

Thirteen patients were scanned by a second-year resident, 124 by a third-year resident, and three by a fourth-year resident (Fig. 1). Thirteen patients were scanned by fellows and seven by first-year attending physicians (who were chief residents in the first half of the study). Seventy-four scans were done from 8 AM to 5 PM. Fifty-six patients were scanned at night and 30 over a weekend. Abdominal scanning was done in 21 patients, transvaginal in 113, and both modalities in 21. The scanning lasted <5 minutes in 46 patients, 5 to 15 minutes in 104, and >15 in 10 (Fig. 2). The reasons for the patients being scanned <5 and >15 minutes are presented in the Results section. The diagnosis before and after the scans on the scanning days is shown in Figs. 3 and 4. Sixty-eight patients had pregnancy problems, 70 patients were scanned to rule out ectopic pregnancy, and there were 10 patients with various pelvic disorders. The data were processed by the statistical package SSP version 4 (SPSS Inc., Chicago) and displayed by the Harvard Graphics package (Harvard Graphics SPC Software, Mountain View, Calif.).

Results

One hundred sixty-eight patients were scanned on the scanning days. Of these patients 83% had suspected pregnancy problems or ectopic pregnancies (Fig. 3). After the scans, 53 intrauterine pregnancies were diagnosed, eight ectopic pregnancies were found, a normal pelvis was diagnosed in 26 patients, and six ovarian

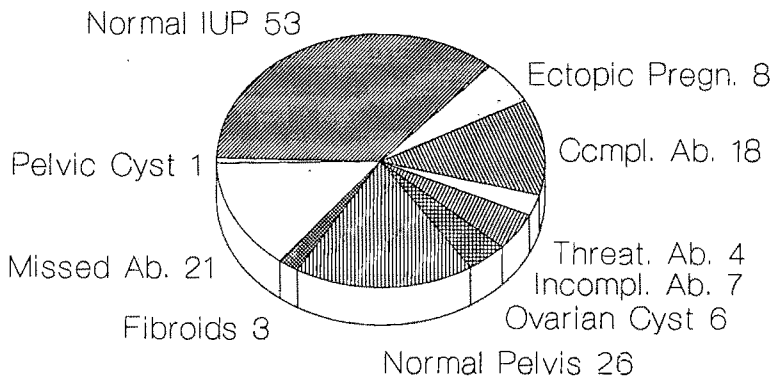


Fig. 4. Diagnosis after scans (all cases) (n = 166).

cysts and one pelvic inclusion cyst were seen. Fifty-two various pathologic conditions of pregnancy were diagnosed, such as complete, incomplete, threatened, and missed abortions (Fig. 4). The breakdown of the diagnoses in the control group (n = 61) was similar to the diagnoses in the scanned group. Most of the patients in whom the examination lasted <5 minutes had suspected pregnancy problems or suspected ectopic pregnancies. The results of the short scan showed normal pregnancies and pathologic pregnancies in most of these cases and only one ectopic pregnancy with a live fetus. All these diagnoses could be made in <5 minutes by using transvaginal ultrasonography. Ten patients were scanned >15 minutes. Seven of them had suspected ectopic pregnancies, two suspected fibroids, and one ovarian torsion.

On the scanning days 76 patients were discharged sooner, 14 patients were admitted earlier, and 14 received surgical treatment sooner. In 53 patients the residents thought that the scan did not change the management of their care. To ascertain that the scanned and the nonscanned patient populations were the same, we looked at the 61 patients who were not scanned. In 43 of the 61 the initial working diagnosis was changed after further diagnostic tests were performed. To answer whether the scans helped the examiner, we evaluated 160 responses. In 121 patients the residents believed management was quicker than usual; in five patients, establishing the diagnosis took a longer time, and in 34 patients the residents thought that the time required to make the diagnosis with or without a scan would have been the same. For the question of whether ultrasonography made the diagnosis more complicated, easier, or the same as without the scan, only four of the 159 questionnaires reflected a more complicated diagnostic process with the scans. In 126 the diagnosis was made easier, and in 29 the residents thought that ultrasonography made no difference in establishing the diagnosis. In 158 questionnaires the residents evaluated their own confidence level. One hundred fifteen felt highly confident with the ultrasonographic examination done in the emergency room, 33 had intermediate and nine low confidence, and in one case the resident was not sure about the level of confidence.

The time spent by the patient in the emergency suite before the diagnosis was reached was 34 minutes in the study group and 215 minutes in the control group (Fig. 5). This was found to be a significant difference at the level of $p < 0.0001$. To arrive at the diagnosis of ectopic gestation took 40 minutes on the scanning days (n = 8) and 190 minutes on nonscanning days (n = 4) (Fig. 6).

There were six missed diagnoses in the scanned group; one normal pregnancy and one complete abortion turned out to be incomplete abortions. The left adnexal mass was diagnosed during surgery as an appendiceal abscess. Three ectopic pregnancies were missed: One was diagnosed as a complete abortion, in the second a normal pelvis was suggested, and the third was labeled as a missed abortion. None of the ectopic pregnancies ruptured, and no patients were discharged home before the correct diagnosis was made, by means of β-human chorionic gonadotropin levels and a repeat sonogram, and treatment was rendered.

Comment

The American College of Obstetricians and Gynecologists issued suggested guidelines for training in ultrasonography.[10] An article was presented at a national meeting[1] and a review article was published to emphasize the use of ultrasonography in the delivery room.[11] It was clear that there was a growing use and suggested need for ultrasonography in labor and delivery rooms. The instrumental progress made in residency teaching of ultrasonography was primarily in the domain of obstetrics.[12] In the imaging literature there is no mention of the gynecologic or emergency room use of this important laboratory tool by residents in obstetrics and gynecology. As noted earlier, the published studies are in the domain of abdominal ultrasonography and were performed by radiologists.

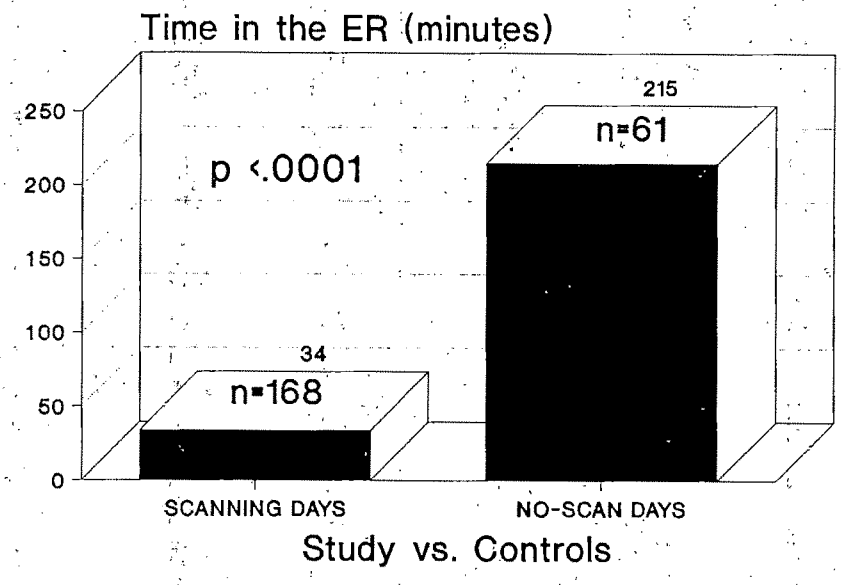


Fig. 5. Time to make the diagnosis.

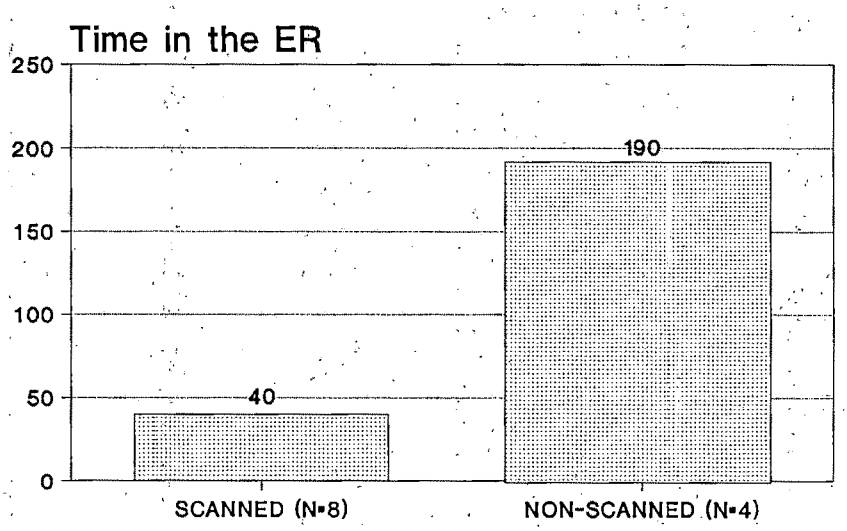


Fig. 6. Average emergency room time (minutes) for ectopic pregnancies.

We reviewed the literature concerning the use of transabdominal ultrasonography in the emergency room, for the management of gynecologic patients. Stabile et al.[3] used high-resolution abdominal sector scanning in the gynecologic emergency room for pregnancy-related diagnoses. In 158 of 624 patients there was no evidence of pregnancy, 60 patients had ectopic pregnancy, and in 406 women the ultrasonographic examination correctly identified intrauterine pregnancy as the cause of vaginal bleeding. Grumbach et al.[4] reported on the emergency room use of computerized tomography and ultrasonography in patients seen in the emergency room with bleeding during pregnancy and other gynecologic problems. Ultrasonography and computerized tomography were considered by these authors as important tools in the workup of many patients treated by the emergency room physician. Shea and Aghababian[5] evaluated the frequency of abdominal and pelvic ultrasonography in the emergency room. Forty-three of 1010 patients who were seen in the emergency room with abdominal or vaginal bleeding required ultrasonography.

It was clear that a study to evaluate the use of transvaginal ultrasonography in the emergency room was needed. The physician seeing obstetrics and gynecology patients in our emergency room is a resident already trained in ultrasonography/ therefore we chose the obstetrics and gynecology resident physician as our model for this study.

The results of our study emphasize several points in the efficacy and diagnosis of gynecologic emergency room illness and the cost of reaching a diagnosis.

First, of the 167 patients who were scanned on scanning days, 83% had pregnancy-related diseases, such as "rule out ectopic" and other problems in pregnancy. On the nonscanning days the diagnoses were similar.

In one third of these cases (44/138 patients) the final diagnosis was aided by a scan completed in ≤5 minutes with ultrasonography. The most important subset of the pregnancy-related problems are the patients with suspected ectopic pregnancy. A closer look at this patient population revealed that only seven of 70 patients with suspected ectopic pregnancy had the condition. The patients in the study group were diagnosed relatively rapidly (40 minutes as opposed to 190 minutes in the control group) as having an ectopic gestation. This evaluation was longer on the nonscanning days because of waiting for a quantitative pregnancy test and an ultrasonography scan, off site and at another time. An additional ectopic gestation was found in a patient with a suspected ovarian cyst during the bimanual examination preceding the scan.

The second important aspect of the study was the statistically significant time difference for arriving at the final diagnosis between the scanned group and the control group. The faster turnover in the emergency room and the shorter time until definitive treatment can be started is an achievement appreciated not only by hospital staff but also by residents themselves who can use the excess time generated. Our initial idea was to measure the efficiency of ultrasonography in the emergency room by looking at the number of unnecessary admissions occurring on nonscanning days. However, one must realize that even on nonscanning days the patients had gotten the necessary laboratory tests and ultrasonography scans at an off-site location. Therefore regardless of the diagnostic route a correct diagnosis was achieved. Comparing the number of admissions in the scanned and nonscanned group would not necessarily reflect the difference in the diagnoses. The same is true if we look at the necessary or unnecessary procedures that occurred. The one area that is indicative of the performance of scans in the emergency room is the time difference between the two groups, that is, the time at which the final diagnosis was reached.

The third and last point is the issue of the missed diagnoses. As with every laboratory tool, the ultrasonography examination also has its misdiagnoses. A total of six missed diagnoses were noted in the scanning group. Three of them concerning abnormal intrauterine pregnancies seem insignificant because they did not jeopardize the immediate treatment given to the patient. Of more concern are the misdiagnosed ectopic pregnancies. None was a bleeding or ruptured tubal ectopic pregnancy. Had that been the case, the free culde-sac fluid would have been recognized at once by the residents who are specially trained for this occurrence. None of these three patients was discharged because they had a positive β-human chorionic gonadotropin test result and there was no intrauterine pregnancy. The suspicion of an ectopic pregnancy was therefore maintained. The correct diagnosis was made during subsequent procedures (curettage and laparoscopy).

Transvaginal ultrasonography contributed valuable information to the diagnostic process in 41% of our study cases by changing the initial diagnosis and bringing about a correct diagnosis.

In summary, the use of ultrasonography performed by experienced obstetrics and gynecology residents was tested. The analysis of the study group and control group showed differences in (1) the speed of the diagnostic process and (2) the accuracy of the diagnosis in favor of the scanned group.

There were six missed diagnoses in the scanned group but no ill effects on the patients. In spite of a few complicated and lengthy scans the residents expressed a high confidence level in operating ultrasonography equipment in the emergency room.

Therefore we conclude that the residents can efficiently use transvaginal ultrasonography in the emergency room. This transvaginal ultrasonography is in addition to the bimanual pelvic examination and should be performed subsequent to the bimanual examination. The emergency room ultrasonography in general, transvaginal ultrasonography in particular, is important because (1) it shortens the time spent in the emergency room, not only for the patients but also for the managing residents, and (2) it contributes to a correct and quick diagnosis in the majority of the patients.

The statement of the American College of Emergency Physicians and the initial report from the American Institute of Ultrasound in Medicine Ad Hoc Committee on Ultrasound in Emergency Medicine clearly states that ". . . it is the responsibility of the ultrasound physicians (Radiology, Cardiology, Obstetrics, etc.) to provide the ultrasound service . . . on a timely 24-hour seven days a week basis. . . ."

For quick and efficient patient care the use of ultrasonography (including a vaginal probe) in the emergency room, operated by the resident on call, should be encouraged.

We thank the resident staff of our department whose collaboration and hard work in generating the data helped bring it all together.

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Ultrasonographic dating of pregnancy causes significant errors in Down syndrome risk assessment that may be minimized by use of biparietal diameter–based means

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OBJECTIVES: Gestational dates assessed by ultrasonographic measurement of fetal dimensions are usually quoted in terms of complete weeks because the uncertainty of ultrasonographic measurement is approximately ±7 days. This study examines the effect of ultrasonographic dating on Down syndrome risk assessment.
STUDY DESIGN: The effect of small changes in measured biparietal diameter resulting in a change in estimated gestational week and the benefits of a more precise measure of fetal gestational age (raw biparietal diameter) are examined mathematically.
RESULTS: If maternal serum α-fetoprotein and human chorionic gonadotropin are used to assess Down syndrome risk, risks assessed with ultrasonographically determined dates may be ≤45% too high for fetuses with biparietal diameter at the lowest end of the size band and ≤22% too low with biparietal diameter at the top of the size band. If the results for maternal serum α-fetoprotein, human chorionic gonadotropin, and unconjugated estriol are used, these figures become ≤150% and ≤60%, respectively.
CONCLUSION: If ultrasonography is used to assess gestational age, the raw biparietal diameter and not the estimated week of gestation must be used to derive means for calculation of multiples of the mean.
(AM J OBSTET GYNECOL 1992;166:872-7.)

Key words: Trisomy 21, multivariate risk screening, α-fetoprotein, human chorionic gonadotropin, unconjugated estriol

In the majority of hospitals in the United Kingdom the gestational duration is calculated from the date of last menstrual period (LMP); this date is checked by ultrasonographic measurement of the crown-rump length or biparietal diameter (BPD). In some hospitals the LMP date is used as the "gold standard" and is only altered by the results of the ultrasonographic examination if there is a discrepancy in dates of ≥2 weeks. Other hospitals use the BPD-derived dates as the final arbiter of gestational age. Unfortunately, it is not possible to estimate exact dates from BPD measurements (i.e., the relationship between BPD and true gestational age is continuous, but because of analytic imprecision and biologic variation the technique can only give an accuracy of prediction of ±7 days[1]). Consequently, gestational dates derived from BPD data are invariably

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quoted in terms of complete weeks, such that a band of BPD sizes corresponds to a single week of gestational age; this inevitably results in imprecision at the edges of the size band. This loss of precision, however, is not confined to interpretation of BPD measurement because gestational age calculated from LMP is invariably rounded up or down to the nearest whole week. In our laboratory 77% of the samples received for Down syndrome screening are from women who had been ultrasonographically assessed before blood sampling; 90% of these women have gestational age quoted in whole weeks.

We investigated the difference between Down syndrome risks calculated with multiples of the mean for maternal serum α-fetoprotein (AFP) and human chorionic gonadotropin (hCG) levels with means based on BPD-derived gestational age (gestational week) and means based on raw BPD data. We estimated inaccuracies resulting from addition of a third analyte, unconjugated estriol.

Methods and results

The screening data of 4605 women routinely screened for Down syndrome–associated pregnancy between January and November 1990 were examined. After exclusion of 1060 data sets from women with multiple pregnancies, women who had not been scanned, and women whose data sets were incomplete, and exclusion of 523 results from women who had not been scanned on the same day as the blood sample was collected for screening, there were 3022 complete data sets. A complete data set included the mother's age at expected date of delivery, BPD measured outer table to outer table, AFP, and hCG.

Table I shows the size bands used to determine gestational week. Many laboratories receive ultrasonographically derived gestational age as a decimal value (e.g., 16.1, 15.5). These values are derived from a similar size-band table or by calculation with regression parameters for BPD versus dates. Although decimal values appear to be more accurate, they are just as uncertain as other ultrasonographically derived measurements.

Log transformation of AFP and hCG values resulted in normalization of the data, and therefore least-squares regression of log 10 (AFP) and log 10 (hCG) against raw BPD or gestational week was performed to derive means for AFP or hCG relative to BPD or gestational week. This method generates "regressed mean" values because all the patient results are used to derive the regression parameters. Previous workers[2] have derived "regressed medians" by determining the median for each week individually and then weighted least-squares regression. Medians for weeks 13 through 27 were used. For example, at 16 weeks there were 122 patients; to calculate the regression, the value for the 16-week median was entered into the least-squares regression 122 times. For many of the weeks used, medians were based on <10 patient results. The regressed means are statistically valid because log transformation resulted in a gaussian distribution for the data. Furthermore, only data for weeks 14 through 20 were used to generate regression parameters; regressed means were only considered valid for weeks 15 through 19 (minimum data set was for 15 weeks, $n = 228$). The regression parameters are shown in Table II.

Table I. BPD size bands corresponding to gestational week (outer to outer table)

Gestational week	*BPD size band (mm)*
14	26.1-30.0
15	30.1-34.0
16	34.1-38.0
17	38.1-41.5
18	41.6-45.0
19	45.1-49.0

Table II. Exponential regression coefficients for gestational week or raw BPD against log 10 (AFP) (kilounits per liter) or log 10 (hCG) (units per milliliter)

	Gestational week		*BPD (mm)*	
Coefficient	*AFP*	*hCG*	*AFP*	*hCG*
A	0.7495	2.2797	0.9758	2.0076
B	0.047339	−0.057547	0.01452	−0.017716
r	0.2252	−0.2067	0.2354	−0.2170

Formula for regressed mean, $10^{(A + B \times)}$. x, Week or BPD size; A and B, regression coefficients; r, sample correlation coefficient.

With these regression parameters it is possible to calculate regressed means for BPD-derived, gestational week–based, and BPD-based gestational dating methods (e.g., for a 16-week pregnancy, the regressed mean for AFP is $10^{[0.7495 + (16 \times 0.047389)]} = 32.1$ kU/L). These means are then used to calculate the multiples of the mean (usually given as multiples of the median), which are required for the multivariate method of calculation of Down syndrome risk factors.[3] Regressed means for gestational weeks 15 through 18 and for extremes of BPD size bands are shown in Table III. The BPD size band extremes are the points on a BPD size band at which a minimal change in measured BPD (0.1 mm) is sufficient to change the estimated week of gestation (e.g., 34.0 mm corresponds to 15 weeks and 34.1 mm to 16 weeks). There was no significant difference between means derived for gestational week and locally derived medians.

To examine the effect on screening of varying the

Table III. Calculated means for each week of gestation and for extremes of BPD size bands

Means based on gestational week			*Means based on BPD*		
Week	*AFP (kU/L)*	*hCG (U/ml)*	*BPD (mm)*	*AFP (kU/L)*	*hCG (U/ml)*
			30.1	25.8	29.8
15	28.8	26.1			
			34.0	29.5	25.4
			34.1	29.6	25.3
16	32.1	22.9			
			38.0	33.7	21.6
			38.1	33.8	21.5
17	35.8	20.0			
			41.5	37.8	18.7
			41.6	38.0	18.7
18	40.0	17.5			
			45.0	42.5	16.2

Means calculated with formula shown in footnote to Table II.

Table IV. BPD-based multiples of the mean calculated for AFP and hCG values corresponding to week-based multiples of the means of 0.5, 1.5, and 2.5 for a 16-week–gestation pregnancy

	BPD-based multiples of the mean			
	AFP		*hCG*	
Week-based multiples of the mean AFP + hCG	*BPD 34.1*	*BPD 38.0*	*BPD 34.1*	*BPD 38.0*
0.5	0.542	0.476	0.453	0.530
1.5	1.627	1.429	1.358	1.590
2.5	2.711	2.381	2.263	2.650
Age difference from week-based multiples of the mean (%)	+8.4	−6.5	−9.4	+6

method of calculating the mean, a series of values were tested with the means shown in Table III. These were derived from gestational week or extremes of BPD size band with the regression parameters in Table II. AFP and hCG values were chosen such that with week-based means the multiples of the means would be 0.5, 1.5, and 2.5, assuming the gestational week was week 16. With these values, multiples of the means were calculated with BPD-derived means assuming BPD was at each extreme of the size band. These values are shown in Table IV. The multiples of the means were applied to the multivariate screening algorithm[3] with parameters previously described.[2] It should be noted that these parameters were derived from LMP-dated pregnancies; however, because the multiples of the median for both Down syndrome and normal pregnancies remain constant throughout the screening time window, it is unlikely that different dating methods will significantly alter the differential between normal and affected pregnancies. Therefore risk factors calculated with these parameters should be representative. The Down syndrome risk factors calculated with these multiples of the means and the percentage differences between the gestational week–based multiples of the mean Down syndrome risk factor and the BPD-based multiples of the mean Down syndrome risk factors are shown in Table V.

Finally, the effect of a 0.1 mm change in BPD, which results in a change from one size band to another, was examined. Risks were calculated with week-based means to calculate multiples of the means for values corresponding to 0.5, 1.5, and 2.5 multiples of the means for BPD equal to 38 and 38.1 mm. These multiples of the means are shown in Table VI; the week-based multiples of the mean Down syndrome risk factor and percentage difference between risks at gestational weeks 16 and 17 are shown in Table VII.

Comment

Biochemical screening for Down syndrome in pregnancy has only recently become possible. The first steps were reported in 1984 when the association between low maternal serum AFP and Down syndrome was reported.[4, 5] Later the inclusion of maternal age[6] introduced the concept of risk screening with a cut-off designed to select patients requiring amniocentesis. This technique was taken up rapidly, and by 1988 approximately 1 million pregnancies in the United States were

Table V. Risk for woman at 16 weeks' gestation who will be 20 years of age at expected date of delivery

AFP multiples of the mean	*hCG multiples of the mean*					
	0.5	*% Difference*	*1.5*	*% Difference*	*2.5*	*% Difference*
0.5						
BPD_{low}*	6,386		858		266	
		+38.0		+43.0		+45.3
Week	4,629		600		183	
		−17.6		−19.3		−20.2
BPD_{high}†	3,814		484		146	
1.5						
BPD_{low}*	64,664		5,994		1,565	
		+42.2		+47.5		+50.0
Week	45,465		4,062		1,043	
		−18.8		−20.5		−21.3
BPD_{high}†	36,873		3,228		821	
2.5						
BPD_{low}*	185,914		14,502		3,496	
		+44.1		+49.5		+52.0
Week	129,017		9,699		2,299	
		−19.5		−21.2		−21.9
BPD_{high}†	103,745		7,643		1,794	

Risk factors shown equal $1:n$, which implies that for n trials there is one chance of a specified event occurring. Increased or decreased risk indicates that there is greater or less, respectively, probability of event occurring. A risk of $1:200 > 1:300$; a risk of $1:300 < 1:200$. Risk factors are shown to six significant figures to emphasize differences. Risks generated in screening would not be reported to this accuracy. All multiples of the mean are week-based. Multiples of the mean used for BPD_{low} and BPD_{high} are those in Table IV.

*34.1 mm.

†38.0 mm.

Table VI. Week-based multiples of the mean calculated for AFP and hCG values corresponding to BPD-based multiples of the mean of 0.5, 1.5, and 2.5 fetus with BPD of 38.0 to 38.1 mm

	Week-based multiples of the mean			
	AFP		*hCG*	
BPD-based multiples of the mean AFP + hCG	*Week 16*	*Week 17*	*Week 16*	*Week 17*
0.5	0.525	0.471	0.472	0.54
1.5	1.575	1.412	1.415	1.62
2.5	2.625	2.353	2.358	2.70
Age difference from BPD-based multiples of the mean (%)	+5	−5.8	−5.6	+8

screened annually.[7] This screen allowed detection of only 25% of affected fetuses in women aged <35 years.[8] At this time it was discovered that other serum analytes were also affected by the presence of a Down syndrome affected–fetus (hCG,[9] unconjugated estriol[10, 11]). The addition of these analytes to the screening method improved the detection rate to 63%.[12] It is thus clear that the addition of the extra analytes significantly improved the screen; however, it must be emphasized that this test is still in its infancy and its precision may be further improved.

One problem with the screen is interpretation of results partly because there are a number of methods for calculation of risk (discriminant function analysis,[13] AFP/hCG ratio,[14] multivariate gaussian distribution function[2, 3]) and partly because risk prediction is a new concept for many clinicians. Furthermore, the concentrations of the analytes vary throughout pregnancy; some method of assessing gestational age must be used to allow standardization of the measured concentrations. The original description of Down syndrome screening with the multivariate method included gestational dating by LMP.[2] This method is problematic because of poor recall of LMP date and variations in cycle length. Similarly, estimation of gestational age by ultrasonography is sometimes inaccurate because of variations in fetal growth rate and poor recall of LMP date, both of which render collection of accurate data relating BPD to gestational age impossible. This inability to accurately date a pregnancy by LMP or BPD

Table VII. Risks calculated for a woman with BPD of 38.0 to 38.1 who will be 20 years of age at expected date of delivery

AFP multiples of the mean	*hCG multiples of the mean*					
	0.5	*% Difference*	*1.5*	*% Difference*	*2.5*	*% Difference*
0.5						
Week 16	5,607		742		229	
		−35.4%		−38.5%		−40.2%
Week 17	3,623		456		137	
1.5						
Week 16	56,007		5,114		1,326	
		−38.0%		−41.0%		−42.3%
Week 17	34,727		3,017		765	
2.5						
Week 16	160,195		12,308		2,946	
		−39.2%		−42.1%		−43.4%
Week 17	97,476		7,125		1,667	

Multiples of the mean used for weeks 16 and 17 are those in Table VI. All multiples of the mean are BPD-based. Risk factors shown are 1 : n. Risk factors are shown to six significant figures to emphasize differences. Risks generated in screening would not be reported to this accuracy.

may partially explain previous reports that BPD dating does not improve screening efficiency in AFP screening for Down syndrome[15] and that combined LMP and BPD dating does offer some improvement in neural tube defect screening.[16]

Further complications include numerous protocols for relating BPD to gestational week, differences in the fetal growth rate between races, and differences in methods of measuring BPD (inner to outer table, outer to outer table).[15, 17-22]

Our investigation avoided many of the above pitfalls because a single BPD/age conversion protocol was used, all measurements of BPD were performed by the same method (outer to outer table), and the population of the county Gwent is mostly racially homogeneous.

The results in Table V clearly demonstrate that if a risk is calculated with the two variables (AFP and hCG) and if gestational week is estimated by ultrasonographic examination the risk generated may be significantly at variance with the risk factor calculated with BPD-derived means. Thus if the fetal head is at the lowest end of the size band it is possible that the risk calculated is too high by ≤45%, which may result in an unnecessary referral for amniocentesis. Similarly if the fetal head is at the top of the size band the risk calculated may be up to 22% too low, resulting in a failure to refer for amniocentesis.

Table VII provides further evidence that basing multiples of the mean calculation on gestational week derived from ultrasonography is not effective. A change in BPD of only 0.1 mm, resulting in a change of size band, is sufficient to change the calculated risk by 40%.

These results only cover the effect of two screening variables. The effect of three screening variables (i.e., including unconjugated estriol) can be estimated by conservatively assuming a ±5% variation between the unconjugated estriol week–based and BPD-based means and applying the resulting values to the risk calculation algorithm as before. This results in an estimate that, with three variables and gestational week–based means, the error may be 55% to 150% for fetal head sizes at the lowest end of the size band and 25% to 60% at the top of the size band (data not shown).

Furthermore, these estimates of errors in risk do not take analytic errors into account. The error for ultrasonographic measurement of BPD was reported as approximately 1.5 mm in 1974[23] which, at a BPD of 34 mm, corresponds to 4.5%. Comparative studies in 1984 gave an absolute error of 1.7 mm for an object 51 mm in diameter (3.3%).[24] Newer ultrasonography machines may be more accurate, but because the above studies did not include observer errors, this is unlikely. Likewise in our laboratory, for AFP at a concentration corresponding to 0.6 multiples of the mean for week 16, the coefficient of variation is 7.8%; for hCG the coefficient of variation is 9.9% at a concentration corresponding to 1 multiple of the mean.

The unavoidable errors from analytic variation can only worsen the situation. Deliberate loss of precision by converting a relatively precise measurement of gestation (BPD) to an imprecise measure (gestational week) can and should be avoided. We therefore believe that if gestational dates are to be checked by ultrasonography, means based on BPD should be used instead of medians based on gestational week derived from BPD. To implement this method of screening will necessitate the coordination of ultrasonographic examination and biochemical sampling so that they are performed on the same day.

Finally, it should be remembered that genetically ab-

normal fetuses may have abnormally small or large heads. Obviously if the defect is gross it will be recognized and appropriate action will be taken; however, minor abnormalities may not be detected. This could affect gestational staging either by BPD alone or by BPD-derived gestational week. To minimize this effect, the ratio between BPD and another ultrasonographic measurement may be effective. The BPD/femur length ratio has already been shown by a number of investigators to be abnormal in Down syndrome. This ratio also shows a trend through gestation that may be standardized for use with BPD.[25]

Because the imprecision of the screen increases exponentially as more analytes are included, the effect of replacing one of the biochemical variables by the ultrasonographic measurement should be examined in addition to the effect of BPD-based means for calculation of multiples of the means. The most likely candidate for exclusion would be unconjugated estriol because, although its decreased concentration in Down syndrome–affected pregnancies is similar to the decrease in AFP, AFP has a second role in screening for neural tube defects. Unfortunately, we do not have access to a sufficiently large population to test the effect on screening efficiency of BPD-based means, but we believe that there is likely to be an improved detection rate and probably a significant decrease in the false-positive rate if BPD-derived means are used.

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Infarction of the uterus from subacute incomplete inversion

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A patient with subacute incomplete uterine inversion was transferred to our hospital when the diagnosis was made 5 days after vaginal delivery. Intravenous injection of sodium fluorescein dye identified a clear demarcation between viable and necrotic myometrium. The superior aspect of the uterus was infarcted; therefore a hysterectomy was performed. (Am J Obstet Gynecol 1992;166:878-9.)

Key words: Uterus, inversion, infarction, fluorescein

Subacute incomplete inversion of the uterus is a rare obstetric complication. We cared for such a patient, in whom the intraoperative administration of intravenous sodium fluorescein dye confirmed the diagnosis of infarction of the uterus, which led us to perform a hysterectomy.

Case report

A 28-year-old woman, gravida 2, para 1, abortus 1, had a vaginal delivery at term complicated by postpartum hemorrhage. She received three units of packed red blood cells because of the hemorrhage, thought to be a result of uterine atony. She was started on a regimen of oral methylergonovine maleate. On the second day after delivery, a fever of 38.3° C and a leukocytosis of 26,200 white blood cells per milliliter developed. She was started on a regimen of intravenous ampicillin.

Abdominal pain persisted, and 1 day later she underwent a pelvic ultrasonographic examination which revealed a 20 × 13 × 13 cm uterus with endometrial fluid "consistent with endometritis." Pain and vaginal bleeding continued, and 2 days later the patient had a pelvic examination done. The fundus could not be palpated abdominally, and the patient was transferred to our hospital.

On admission vital signs were normal and temperature was 37.6° C. Pelvic examination revealed a dusky mass protruding through the cervix and extending to within 3 cm of the introitus. Hematocrit was 32%. She was given intravenous clindamycin and gentamicin. In the operating room, in spite of the administration of halogenated anesthetic agents and intravenous magnesium sulfate and terbutaline, we could not reduce the inversion by a vaginal approach.

A vertical abdominal incision was then made, and we found the fundus to be inverted with both tubes and ovaries drawn into an inversion ring (Fig. 1). The tight ring prevented replacement of the uterus in spite of the use of traction sutures in the uterus (Huntington procedure)[1] combined with elevation of the fundus with the surgeon's hand in the vagina. A vertical incision of the anterior surface of the uterus enabled us to restore the uterus to its normal position, yet the superior aspect of the uterus felt soft and appeared dusky. Suspecting infarction of the uterus from entrapment in the inversion ring, we injected 700 mg of sodium fluorescein dye (10 mg/kg) intravenously and illuminated the uterus with a fluorescent lamp. A clear line of demarcation between viable and necrotic myometrium was evident. Because of the extent of the injury and the established infection of the necrotic uterus, we performed a hysterectomy. The patient's postoperative course was smooth, and she went home 5 days later.

The pathology report on the uterus revealed the lower uterine segment to be firm, tan, and normal for the postpartum state. The transition between viable and dead tissue was distinct. The necrotic area involved the posterior wall of the uterus more than the anterior wall. Microscopic examination indicated extensive coagulative necrosis of the myometrium and acute endometritis and myometritis with occasional bacterial colonies. There were also ischemic changes in areas bordering the regions of coagulative necrosis.

Comment

Cases of inversion of the uterus are classified both by timing in relation to delivery and by extent of the anatomic defect. Cases are termed acute within 24 hours of delivery, subacute from 24 hours to 4 weeks after delivery, and chronic after 4 weeks. With incomplete inversion the fundus extends to the level of the cervix (first degree) or below the cervix but not to the introitus (second degree). In contrast, with complete inversion the fundus extends either to the introitus (third degree) or beyond the introitus, with the vagina inverted as well (fourth degree).[1] In a 9-year review of uterine inversion in our hospital, encompassing >100,000 deliveries, the incidence of inversion was four per 10,000 deliveries. Of these, <4% were subacute, with the remainder being acute. On the other

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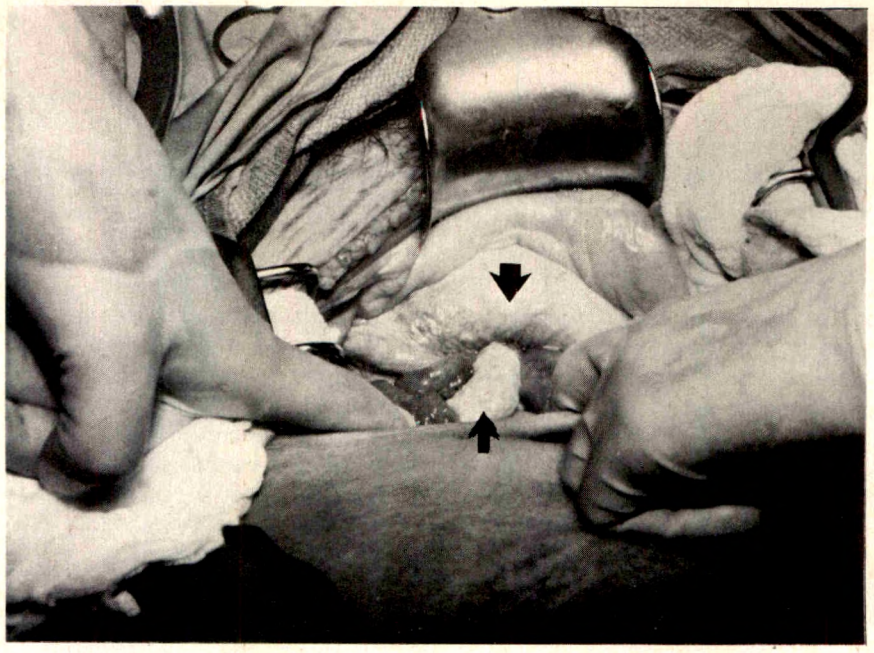


Fig. 1. View of superior aspect of uterus at laparotomy, showing inversion ring *(large arrow)* with both fallopian tubes and one ovary *(small arrow)* drawn into ring. One ovary was caudad to inversion ring.

hand, second-degree incomplete inversion was the most frequent extent of inversion, as occurred in this case.

We chose an anterior hysterotomy incision because of ease of exposure. In contrast, the traditional approach to this problem has been an incision on the posterior surface of the uterus (Haultain procedure),[2] first described at the turn of the century. In addition, vaginal hysterotomy procedures, such as the Spinelli and Küstner operations, have been used with success.[2]

Infarction as a complication of uterine inversion has not been described in recent case-series reports. However, death from gangrene of the uterus has been reported decades ago.[3] In addition, one case of intestinal strangulation, presumably from entrapment in the inversion ring, has been described. The infrequency of infarction as a complication of inversion may reflect the low incidence of inversion, rapid diagnosis, prompt treatment, or a combination of these.

Use of intravenous sodium fluorescein dye confirmed the diagnosis of necrosis of the uterus. Since the early 1940s this technique has been used to determine circulatory failure, ischemia, and necrosis. With an intravenous dose of 10 to 15 mg/kg, all perfused tissues will exhibit fluorescence within 5 to 7 minutes. The technique has been used clinically to determine the viability of bowel during gastrointestinal operations and to guide the extent of excision of infected soft tissue. The approach is simple, safe, and inexpensive; in this case it allowed a definitive diagnosis of infarction to be made during the initial laparotomy, thus avoiding the need for a later hysterectomy.

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The primary application of three-dimensional ultrasonography in obstetrics

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To date, no report of three-dimensional ultrasonography in obstetrics has been available in the literature. In this study we describe the primary experience with three-dimensional ultrasonography for the prenatal evaluation of nine fetuses between 21 and 33 weeks of gestation. We found that it provides more information for clinical diagnosis than the traditional two-dimensional ultrasonography. The following advantages are notable: (1) the anatomic area of interest can be located first with the conventional real-time two-dimensional mode, then switched to reveal three-dimensional images, instantly; (2) three images are exhibited concurrently; (3) each of the three images can be arrayed separately and scrolled to search for the area of interest within the scanned volume; (4) the three-dimensional ultrasonography can be equipped with Doppler color flow mapping for the study of the fetal cardiovascular system. In conclusion, we believe that three-dimensional ultrasonography can greatly strengthen diagnostic potential, which deserves obstetricians' attention. (Am J Obstet Gynecol 1992;166:880-6.)

Key words: Three-dimensional, ultrasonography, obstetrics

The application of real-time ultrasonography has played a very important role in modern obstetrics. Traditional real-time two-dimensional ultrasonography is usually position dependent and gives only a linear observation of the structures of the fetuses. However, there are several disadvantages, as a result of oligohydramnios, movable status of the fetus, acoustic shadow caused by skeleton, or trunk and extremities of the fetus that are improperly positioned for observation. It is difficult to scan the structures of the fetus in detail, especially complex lesions of the central nervous and cardiovascular systems.[1] With the recent introduction of three-dimensional ultrasonography, in which three continual different planes are displayed simultaneously, we have attempted to resolve the disadvantages of the traditional two-dimensional echo to get as much accurate information as possible. In this study nine fetuses were examined systemically with both two-dimensional and three-dimensional ultrasonography, including a fetus with hydronephrosis and hydroureter.

Material and methods

Nine fetuses between 21 and 33 weeks of gestation were studied. Before three-dimensional ultrasonographic examination (Kretz Combison 330, 3.5 MHz, Zipf, Austria), each fetus underwent the traditional two-dimensional examination (Aloka 650, 3.5 MHz, Tokyo) in which the following parameters were examined: biparietal diameter, orbital diameter, interorbital distance, chest circumference, abdominal circumference, and femur length. Further examination of the fetal anatomy was done in detail for detection of malformation of the central nervous, cardiovascular, gastrointestinal, genitourinary, and skeletomuscular systems. We paid special attention to the fetal head and heart with three-dimensional ultrasonography for analysis of these complex structures in all spatial planes.

According to the manufacturer's manual, three-dimensional ultrasonography is characterized by a special transducer performing two-dimensional scan, sweeping slowly across an arc of 90-60 degrees. The transducer produces continual two-dimensional images a millimeter apart, across an arc of 90-60 degrees. The entire scanned volume can be stored in a memory system, a procedure that takes about 3.5 seconds. We can view the scanned volume simultaneously in three different planes that are perpendicular to each other; these planes are the B plane, C plane, and O plane, representing the longitudinal, transverse, and horizontal sections (Fig. 1). Within the scanned volume, images confined in the three planes can be moved millimeter by millimeter, and their relative positions are indicated with reference lines at the other two planes on the monitor. Any desired section can be displayed, within the scanned volume, by three position controls. The

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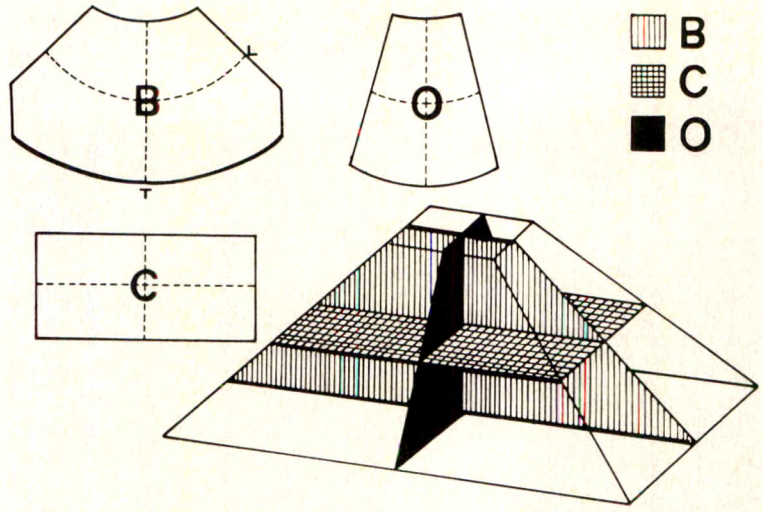


Fig. 1. Within scanned volume, three different planes, *B*, *O*, and *C* planes, perpendicular to each other are illustrated. These planes are consistent in orientation in all subsequent illustrations. T line indicates transverse line of *B* plane. L line is longitudinal line of *B* plane.

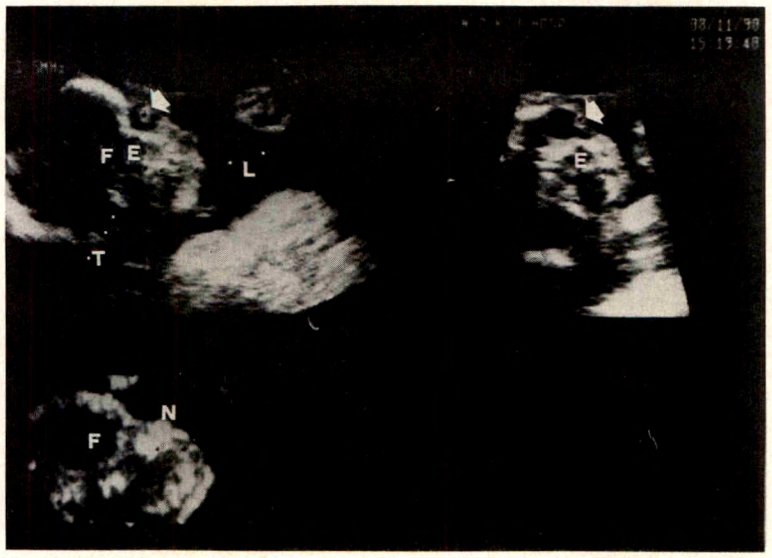


Fig. 2. Fetal face demonstrated well in B plane. Bilateral orbital cavities in O plane were visualized as T line *(T)* was scrolled to midorbital position. Midsagittal view of fetal head in C plane was displayed while L line *(L)* was moved to midface position. *E*, Ethmoid sinuses; *F*, frontal lobe; *N*, nose; *arrow*, lens.

scanned volume may consist of 30 to 60 sections of two-dimensional images about a millimeter apart, depending on the scan angle and depth in one scanned volume. All of these sectional images are presented independently of each other; thus this is a promising technique to analyze the complex anatomy of the fetal head and cardiovascular system.

The three-dimensional ultrasonography is displayed with a 60- or 90-degree sector. The maximum penetration depth of the VWP3.5 3D-Voluson transducer is 210 mm at a transmitting-receiving frequency of 3.5 MHz. The frame rates are 5 to 15 frames/sec at a 90-degree scan angle and 5 to 20 frames/sec at a 60-degree scan angle, depending on the width of sector display and the length and pulse repetition frequency. The power output (spatial-peak temporal average) for three-dimensional ultrasonography is 51 mW/cm^2 measured in water.

Results

With the introduction of three-dimensional ultrasonography, it is feasible to view images of successive fetal anatomic divisions in three different planes simultaneously. Therefore the three-dimensional technology

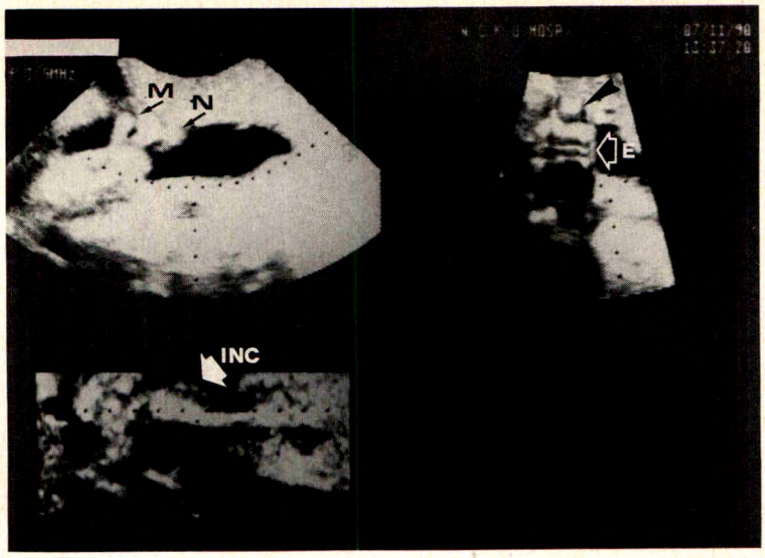


Fig. 3. Coronary view of fetal nose *(N)* and mouth *(M)* seen in B plane. Although orbital cavity was invisible, ethmoid sinuses *(E)* could be seen by scrolling T line to midorbital level. Inferior nasal concha *(INC)* was shown in C plane. *Arrowhead*, Eye.

greatly increases diagnostic potential in obstetrics, with the benefit of its ultimate three-dimensional demonstration of the complicated structures of the fetus. In our study only one fetus with hydronephrosis was detected. The other eight fetuses appeared grossly normal, and serial evaluations of the fetal organs were shown as successive views with the 3.5 MHz transducer.

Fetal head

Fetal face. The fetal face was easily imaged with the two-dimensional real-time mode (Fig. 2, B plane). The bilateral orbital cavities were displayed in the O plane with clear margins by scrolling the transverse line (T line) of the B plane to the midorbital position. Of interest is the ease in observing the midsagittal view of the fetal head, whereas the longitudinal line (L line) of the B plane was moved to the midface position (Fig. 2, C plane). The outlines of the fetal nose and upper and lower lips, as well as the frontal lobe, parietal lobe, occipital lobe, and petrous region, were shown. Similar diagnostic excellence is implied by Fig. 3, in which we cannot see the orbital cavities and ethmoid sinuses in the B plane, yet they do appear in the O plane, through storage of the scan geometry. Because of problems with vertical resolution, the oral cavity and tongue cannot be seen as clearly as the inferior nasal concha and inferior meatus in the C plane (Fig. 3).

Bilateral parietal plane. The axis scan over the bilateral parietal level reveals the complex structures in this volume, including the frontal lobe, temporal lobe, cerebral falx, cavum of septum pellucidum, third ventricle, thalamus, and hippocampus[2] (Fig. 4). Of interest is the demonstration of the corpus callosum in the C plane and the trigone of the lateral ventricle in the O plane, both of which are not always visualized because of a position of the fetal head that is unattainable by conventional two-dimensional echo.

Cerebellum and cervical vertebrae. The coronary section was transmitted obliquely through the fetal head to show the parietal lobe, ambiens, cerebral peduncle, tentorium cerebelli, cerebellum (cerebellar hemisphere and vermis), medulla oblongata, and then the cervical spine from the upper to the lower part of this plane. The cisterna cerebellomedullaris and the sagittal section of the atlantooccipital joint could be displayed in detail, separately.

Cardiovascular system. With the development of high-resolution ultrasonography, we can visualize the fetal heart as early as possible. However, cardiac lesions, such as small septal defects, valvular anomalies, and abnormalities of pulmonary venous return, are usually position dependent with varying presentations of the fetus and are difficult to detect with the use of a two-dimensional scan.[1, 3] We use three-dimensional ultrasonography to survey the complex structures in different planes. We believe that three-dimensional ultrasonography can offer information beyond that with the conventional two-dimensional echo in fetal echocardiography. In our clinical practice we displayed several views of importance in fetal echocardiography, such as the four-chamber view, five-chamber view, and several axis views of the great vessels, and then interpreted these views in different planes for further analysis.

Four-chamber view. In this view the four cardiac chambers, atrial and ventricular septa, foramen ovale, and pulmonary vein are demonstrated as usual. We scrolled the T line to the cross section of the thoracic descending

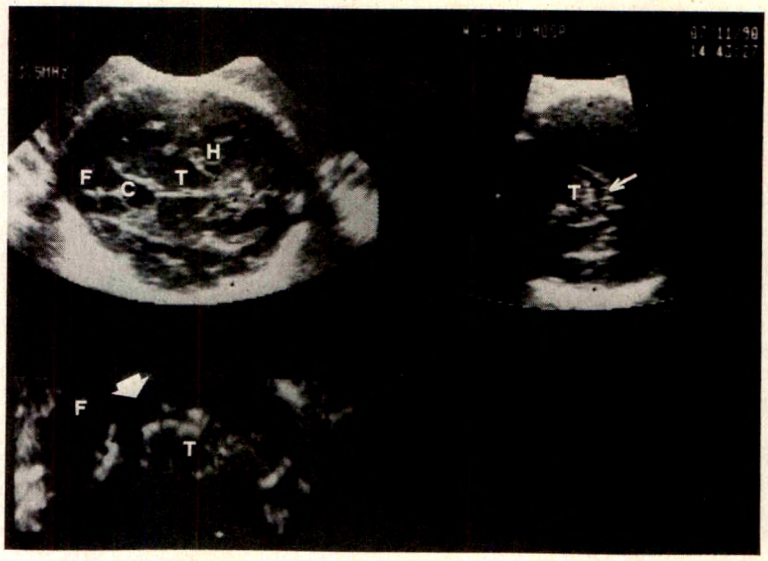


Fig. 4. Axial section at bilateral parietal level revealed frontal lobe *(F)*, cavum septum pellucidum *(C)*, thalamus *(T)*, and hippocampus *(H)* in B plane. Trigone of lateral ventricle *(small arrow)* and corpus callosum *(large arrow)* are shown in O plane and C plane, respectively.

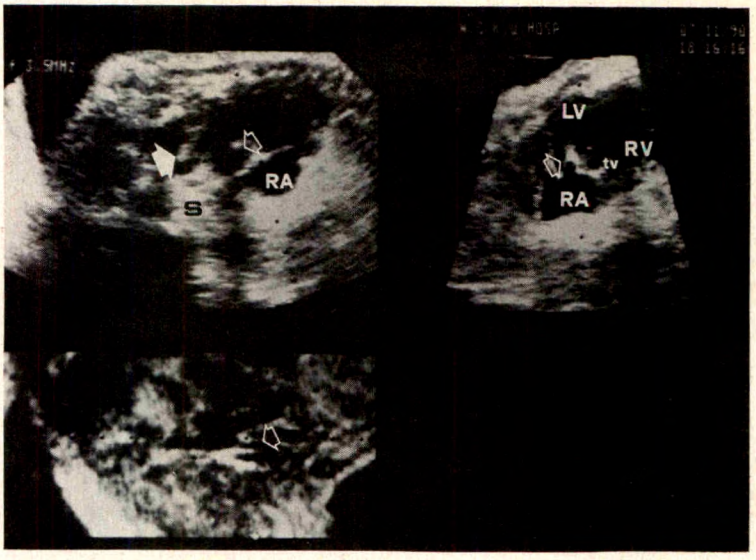


Fig. 5. With slight fetal cephalic tilt of transducer from apical plane, five-chamber view was revealed. Coronary sections of aortic valve *(white halo arrow)* and pulmonary valve *(white arrows)* are indicated. Individual sections of aortic valve were well displayed in O plane and C plane, respectively. *S*, Spine; *tv*, tricuspid valve; *RA*, right atrium; *RV*, right ventricle; *LV*, left ventricle.

aorta; then the long-axis views of the thoracic descending aorta, the superior vena cava, and the pulmonary vein entering the left atrium were demonstrated. The short axis of the ventricles was found by scrolling the L line to this level.

Five-chamber view. With a slight fetal cephalic tilt of the transducer from the plane of the apical view, the ascending aorta constructs the fifth chamber between the right and left atria (Fig. 5, *B-plane*). The coronary section of the cusps of the pulmonary valve and aortic valve could be displayed as the T line was moved to the outlet level of the aorta and pulmonary trunk. The well-shaped aortic valve correlated with the right atrium and right ventricle; the left ventricle was demonstrated in the same B plane at a different location of the T line (Fig. 5, O plane). A similar effect of varying positions of the C plane revealed the longitudinal section of the aortic valves in Fig. 5.

Short-axis–great vessel view. It is essential to interpret the complicated relationship among the great vessels.

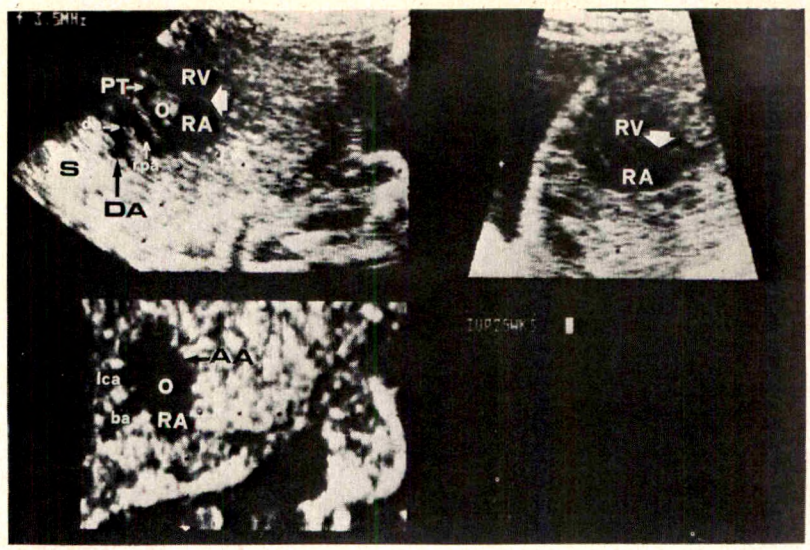


Fig. 6. In this short-axis–great vessel view, right atrium *(RA)*, right ventricle *(RV)*, tricuspid valve *(large white arrow)*, pulmonary trunk *(PT)*, ductus arteriosus *(da)*, and right pulmonary artery *(rpa)* were followed in small counterclockwise rotation (B plane). We can see ductus arteriosus joining descending aorta anterior to spine *(S)*. Longitudinal section of right atrium, right ventricle, and tricuspid valve is shown in O plane. More complicated anatomy of ascending aorta *(AA)* and aortic arch with right atrium is revealed in C plane. *O*, Aortic valve; *ba*, brachiocephalic artery; *lca*, left common carotid artery.

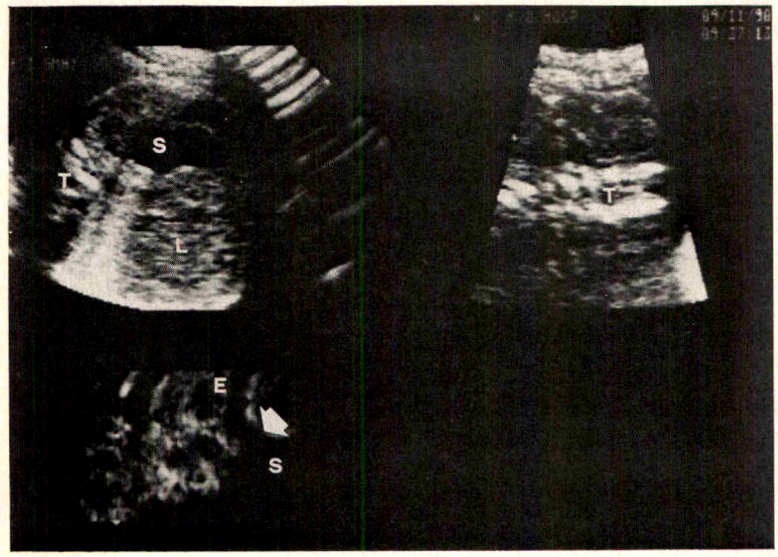


Fig. 7. Transverse section of stomach and liver is displayed in B plane. Well-shaped esophageal-gastric junction *(white arrow)* was unexpectedly demonstrated in C plane. *E*, Esophagus; *L*, liver; *S*, stomach; *T*, spine.

This view shows the right atrium, right ventricle, tricuspid valve, pulmonary valve, main pulmonary artery, ductus arteriosus, and right pulmonary artery arranged in a small counterclockwise rotation (Fig. 6, B plane).[3] The ductus arteriosus enters the descending aorta anterior to the spine. The tricuspid valve was demonstrated well in the O plane. The complex relationship among these greater vessels, including the ascending aorta, left common carotid artery, and brachiocephalic artery, and the aortic valve could be well demonstrated in the C plane.

Umbilical vein and gallbladder. As the ultrasonographic beam transected the fetal abdomen at the bifurcation of the umbilical vein, we could not see the

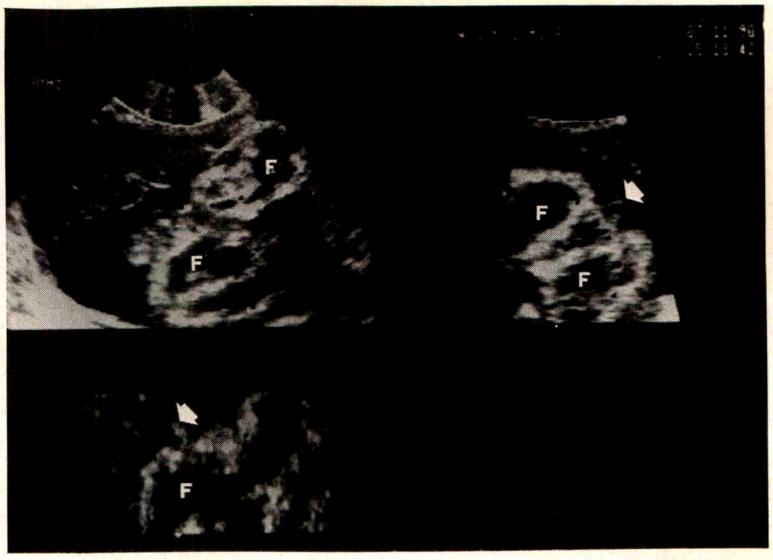


Fig. 8. External genitalia of male fetus could not be identified clearly in B plane, yet we could correctly ascertain male penis *(white arrow)* in both O and C planes. *F*, Femur.

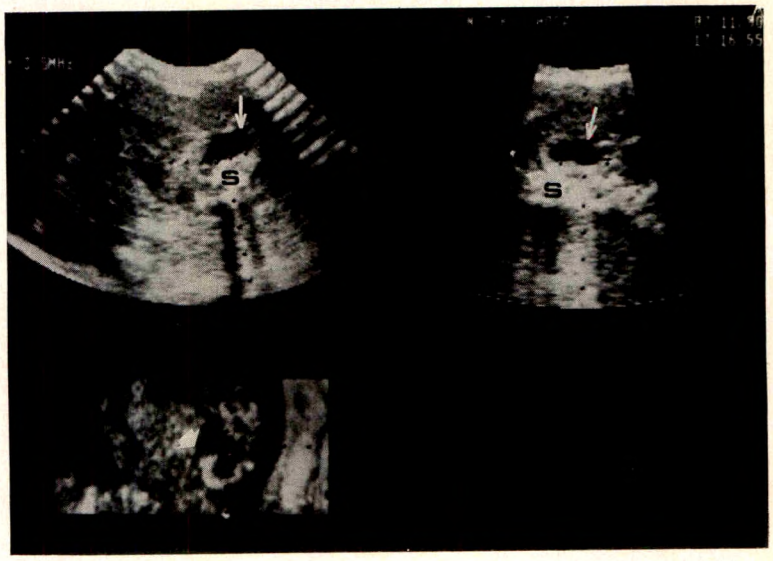


Fig. 9. Hydronephrosis was well demonstrated in three planes. We could see sagittal section of hydroureter *(large white arrow)* in C plane. *S*, Spine.

gallbladder. However, it was visible in the coronary section within the scanned volume.

Esophageal-gastric junction and pylorus. With the fetus lying in a vertex presentation, the B plane displayed the liver, spleen, and cross section of stomach (Fig. 7). Within the scanned volume, the well-shaped esophageal-gastric junction was unexpectedly shown (Fig. 7, C plane). In another case the pylorus was also revealed. In our experience it is uncommon to observe the esophageal-gastric junction and pylorus on the traditional two-dimensional scan. Therefore early prenatal diagnosis of congenital anomalies of the fluid-filled gastrointestinal system will be possible on three-dimensional scan; such diagnosis is difficult after birth because of the poor resolution of the ultrasonographic beam in showing the air-filled organs.

Genitourinary system. Investigation of the genitourinary system has been possible by traditional two-dimensional ultrasonography, yet it is difficult to observe the pelvic-ureter junction and ureterovesical junction except in the presence of hydronephrosis and hydroureter. Three-dimensional ultrasonography allows us to see these fine structures as usual.

It is not always easy to correctly identify the external

genitalia of the fetus because of improper position of the fetus or ambiguity caused by the poor development of exernal genitalia or confusion with umbilical cord. It is easy to resolve this problem with more confidence with three-dimensional interpretation (Fig. 8).

In our study only one case of a fetal anomaly was detected. A 30-year-old woman, gravida 1, visited our obstetric clinic for a routine prenatal examination at 21 weeks of gestation. Ultrasonographic examination demonstrated an intrauterine pregnancy with a single, live fetus in cephalic presentation with normal amniotic fluid volume. A conventional high-resolution, real-time scanner with a 3.5 MHz transducer revealed hydronephrosis and hydroureter of unknown origin. The three-dimensional ultrasonography was performed 2 weeks later, and we could view clearly the anatomic correlation between the kidney, pelvis, and ureter with three-dimensional evaluation (Fig. 9).

Comment

From our initial experience with three-dimensional ultrasonography in obstetrics, we believe that it could become a very important diagnostic tool for the prenatal diagnosis of fetal anomalies for the following reasons:

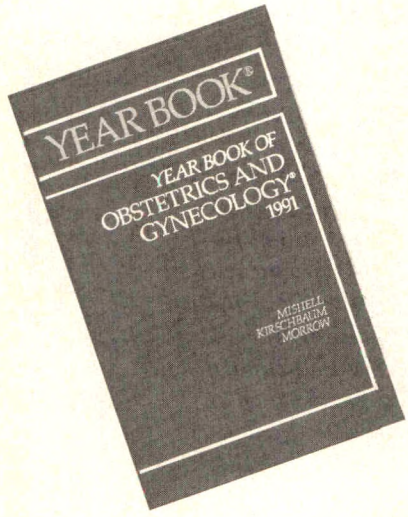
1. The anatomic area of interest is located first by using the conventional real-time two-dimensional mode, followed by three-dimensional images.
2. Three images, the B, C, and O planes, are exhibited concurrently. The C plane is perpendicular to the angle of propagation of the ultrasonographic beam, which is inaccessible by conventional techniques. In our study the corpus callosum, the inferior nasal concha, and the longitudinal section of the aortic valve were demonstrated well in this special plane.
3. All three images can be arrayed separately from each other. This technique provides analysis of structures in different spatial planes.
4. The best potential advantage of three-dimensional ultrasonography is that it can be equipped with Doppler color flow mapping for the further study of pathophysiologic characteristics of the fetal cardiovascular system.[1]

Congenital anomaly of the fetus has become the leading cause of neonatal death.[4] It is well known that accurate early prenatal diagnosis of malformation may improve fetal outcome. Nevertheless, there are many blind points or unattainable planes in the complex anatomy of the fetus. Therefore it is difficult to make the correct interpretation on conventional two-dimensional scan. It is our opinion that the adjunctive use of a three-dimensional ultrasonographic imaging system with the normal real-time two-dimensional mode can greatly increase diagnostic accuracy in obstetrics. It suits the need for further analysis of the complex structures of the fetus, especially when an anomaly of the central nervous or cardiovascular system is suspected. In conclusion, we believe that three-dimensional ultrasonography can greatly enhance diagnostic potential, which deserves the attention of obstetricians.

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NORPLANT® SYSTEM

levonorgestrel implants

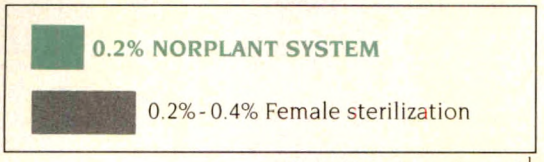


Six reasons why you can feel comfortable recommending it to your patients

Effectiveness comparable to tubal ligation

Although no contraceptive is 100% effective, the NORPLANT SYSTEM is one of the most effective forms of birth control ever developed. The average annual gross pregnancy rate over a five-year period is less than 1%.

Lowest expected and typical failure rates with NORPLANT SYSTEM vs. sterilization during first-year use[1]



Adapted from Trussell J, et al.[1]

Long-term convenience... estrogen-free

NORPLANT SYSTEM, a progestin-only contraceptive, is an excellent option for women desiring a long-term method. Its effectiveness lasts for five years and is not dependent on compliance.

Reversible, with prompt return to previous level of fertility

NORPLANT SYSTEM is reversible at any time; the contraceptive effect ceases when the capsules are removed. In one study, 40% of women wanting to conceive did so within 3 months of removal, 63% by 6 months, 76% by 12 months, and 90% by 24 months.[2]

High patient acceptance despite menstrual pattern changes

NORPLANT SYSTEM causes menstrual irregularities (the most common complaint),* which occur in many women during first-year use and diminish over time. Only 9.1% of patients discontinued during first-year use due to these changes.[3†]

Women who are thoroughly informed of potential menstrual changes and side effects are more tolerant of them when they occur.[4]

Well-received, well-liked by American women

One study of 140 users revealed that 94% were satisfied with NORPLANT SYSTEM, 91% recommended it to their friends, and 74% wanted to use it again.[5]

A 10- to 15-minute office procedure

A local anesthetic and a small 2 mm incision...little or no discomfort during insertion...proper placement should not leave a noticeable scar...in most cases sutures are not needed.



*Serious as well as minor side effects may occur (see prescribing information).

†Based on multicenter trials of 2,470 women.

Please see brief summary of prescribing information on adjacent page.

NORPLANT® SYSTEM
levonorgestrel implants

Lasts 5 years...yet is reversible

NORPLANT® SYSTEM

levonorgestrel implants

Lasts five years…yet is reversible

BRIEF SUMMARY OF PRESCRIBING INFORMATION. CONSULT THE PACKAGE LITERATURE FOR FULL PRESCRIBING INFORMATION.

Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed. Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.

Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.

Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.

Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See **Warnings** section and Full Prescribing Information.

NURSING MOTHERS: See **Warnings** section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).

Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis, and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin, levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

CI 4064-1 12/10/90

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Cellular immunity of patients with recurrent or refractory vulvovaginal moniliasis

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Cellular immunity was studied in 73 patients with recurrent vaginal moniliasis and 37 healthy controls, by skin testing with the multitest CMI kit and *Candida* antigen, with measurement of lymphoblastic transformation to phytohemagglutinin, antigens of *Candida albicans,* mumps, and streptokinase. Eighteen patients (24.7%) had a hypoergic or anergic response to *Candida* antigen on skin testing versus two controls (5.4%), $p = 0.01$. Overall, the patient's lymphoblastic proliferation to mitogen and various antigens was not significantly different from that of the controls. However, a subgroup of younger women (19 to 29 years old) had impaired responses to *Candida* antigen when compared with age-matched controls, 58% versus 17%, $p < 0.005$. Most women with recurrent vaginal moniliasis had normal cellular immunity. (Am J Obstet Gynecol 1992;166:887-90.)

Key words: Cellular immunity, vaginal moniliasis

Most women will experience at least one episode of genital moniliasis in their lifetime. However, these episodes occur infrequently and are easily treated. Well-known factors associated with recurrent or refractory vulvovaginal moniliasis include diabetes mellitus, pregnancy, cellular immunodeficiency, and prolonged or recurrent antibiotic and steroid usage. There is a small group of women with recurrent genital moniliasis who are otherwise healthy with none of the above risk factors. The cause of their recurrent infections is unknown,[1] although a few studies suggest that some of these women may have a mild selective defect in cellular immunity.[2, 3] The aim of this study was to further verify the cellular immune abnormality in a larger group of patients by comparing them with a healthy control group.

Patients and methods

This study was approved by the clinical trials committee, and informed written consent was obtained from all patients and volunteers.

Patient population. Patients were selected for the study if they had four or more microbiologically proved episodes of vulvovaginal moniliasis in the past 12 months and no evidence of diabetes mellitus, chronic underlying illness, or long-term usage of steroids or antibiotics. Patients were referred to our clinic by gynecologists or community family physicians.

Volunteers. We selected healthy female volunteers who had no history of vaginal moniliasis or may have had an occasional episode ≥2 years before entry into the study. Control women selected were between the ages of 19 and 50 years, to match the ages of patients enrolled in the study.

Assessment. A detailed history and complete physical examination were done on all patients and volunteers, including a vaginal examination. Laboratory tests included complete blood cell count, white blood cell count and differential, fasting or 2-hour postprandial blood glucose, serum creatinine, calcium and phosphorus, bilirubin, alkaline phosphatase, serum alanine aminotransferase, and urinalysis. Microbiologic assessment included cultures from the vulva, vagina, mouth, and rectum for yeast.

Cellular immunity. Cell-mediated immunity was assessed by skin test response to the multitest CMI kit (Institut Merieux, Lyon, France) and to a 0.1 ml intradermal injection of fresh *Candida albicans* antigen (1:100 dilution). The response to the multitest CMI skin test was classified as normal (>5 mm), hypoergic (total score <5 mm), or anergic (0 score). A positive reaction to *C. albicans* was read as ≥5 mm of induration. Both tests were read at 48 hours after the inoculation.

Lymphoblastic transformation studies were done on separated lymphocytes (Hypaque Ficoll technique) as previously described[4] and included the mitogenic response to phytohemagglutinin and the response to specific antigens, including *C. albicans,* mumps, and streptokinase. The response to normal media was run as a control. All cultures were carried out in RPMI 1640 medium with 10% fetal calf serum. The blastogenic

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Table I. Demographic Features of patients and controls

	Patients (n = 73)	*Controls (n = 37)*	*p Value*
Age (yr, mean ± SD)	32.3 ± 6.7	28.9 ± 7.2	0.02
Age groups			
19-29 yr	33 (45.2%)	24 (64.8%)	—
30-39 yr	31 (42.4%)	7 (18.9%)	—
40-49 yr	9 (12.3%)	6 (16.2%)	—
Using oral contraceptives	17 (23.3%)	10 (27.0%)	NS
Postmenopausal	1	0	NS
Hysterectomy	1	0	NS
Asthma	1	1	NS
Mild psoriasis	0	1	NS
Eczema	0	1	NS
Venereal warts	1	0	NS
Pyelonephritis (previous)	1	0	NS

NS, Not significant.

Table II. Skin test response

	Patients (n = 73)	*Controls (n = 37)*	*p Value (χ^2)*
CMI (anergic or hypoergic)	14 (19.2%)	1 (2.7%)	0.01
Candida antigen (negative reaction)	18 (24.7%)	2 (5.4%)	0.01

transformation to mitogen and antigen (after 3 and 6 days) was measured by increase in deoxyribonucleic acid synthesis incorporation of tritiated thymidine. A normal response to an antigen was empirically assessed as a greater than twofold increase over the normal medium (unstimulated) and a greater than 50-fold increase for phytohemagglutinin stimulation. A stimulation index was computed by calculating the ratio of response to antigen and mitogen over control media:

$$\text{Stimulation index} = \frac{\text{Response to antigen or mitogen}}{\text{Response to control medium}}$$

The serum inhibitory effect on blastogenic transformation was also assessed in patients and volunteers. The lymphocyte response to the antigens and mitogen was measured in the presence of autologous serum.

Statistical analysis. The characteristics of the two groups, such as mean age, and analysis of continuous variables, such as stimulation index, were compared by use of the Student unpaired *t* test. In comparison of rates of abnormal skin tests (anergic or hypoergic reaction), abnormally low response to the various antigens, use of oral contraceptives, a χ^2 test, or Fisher exact test (one tailed) was used.

Results

Seventy-three patients and 37 volunteers were enrolled in the study. The demographic features are summarized in Table I. There were no significant differences in the use of oral contraceptives or underlying illnesses. The mean age of the patients was statistically higher (32.3 vs 28.9 years) but not considered of physiologic importance.

Results of the skin testing (delayed hypersensitivity) are shown in Table II. Although there was a significant difference in response between the two groups, only 19% and 24% of the patients showed anergy or hypoergic response to CMI or *Candida* antigen, respectively. Fourteen of the patients and one volunteer who had an anergic reaction to CMI and *Candida* antigen (1 : 100 dilution) were restested with a stronger strength of *Candida* antigen (1 : 10 dilution). Of these, only one patient had a negative reaction (<5 mm of induration).

Lymphoblastic transformation. One hundred thirty-eight tests were done in 73 patients, and 71 tests were performed in 37 controls. Thirty-nine patients had repeat testing, 15 patients on four separate occasions, nine patients on three occasions, and 15 patients on two occasions. Thirteen controls had repeat testing, 11 on four occasions and two with two tests.

Table III summarizes the results of the lymphoblastic transformation response to the various antigens and mitogen.

Overall, the patients' lymphoblastic responses to the various antigens and mitogen were not significantly different from those of the controls. The exception was the response to mumps antigen, which was significantly greater in the patients versus controls ($p = 0.01$). However, in a subgroup of patients 19 to 29 years old (Table IV), a greater number had low responses to *C. albicans* antigen when compared with the control group in the same age bracket, 58% versus 17% ($p < 0.005$). The

Table III. Lymphoblastic transformation response

Antigen	*Patients (n = 73)*	*Controls (n = 37)*	*p Value*
C. albicans			
Low response*	39 (53%)	13 (35%)	0.20
Stimulation index, mean ± SD†	7.6 ± 23.3	8.8 ± 19.0	0.35
Mumps			
Low response*	15 (21%)	13 (35%)	>0.1
Stimulation index, mean ± SD†	13.9 ± 21.8	8.3 ± 9.7	<0.01
Streptokinase			
Low response*	15 (21%)	10 (27%)	>0.2
Stimulation index, mean ± SD†	13.3 ± 19.4	10.2 ± 12.9	<0.1
Phytohemagglutinin			
Low response*	16 (22%)	11 (30%)	>0.1
Stimulation index, mean ± SD†	126.4 ± 113.3	107.6 ± 110.5	0.15

*Calculated on the presence of at least one abnormal test result in a given patient.
†Calculated from the total tests performed.

reverse effect was seen in the older age groups where a low response to *C. albicans* antigen was greater but not statistically significant in the control group.

The decrease in lymphoblastic transformation to *Candida* antigen in both patients and controls was often not persistently detected. Fifteen patients and 11 controls with initially low lymphocyte proliferation to *Candida* had repeat testing on four separate occasions. All four test results were abnormal in only one control and no patients; three of the test results were abnormal in five patients and four controls, and only two or fewer of the test results were abnormal in six patients (40%) and six controls (54.5%).

There was no significant inhibitory effect of serum on the patients' or volunteers' lymphoblastic responses. The lymphoblastic response and stimulation index with the various antigens and mitogen were unchanged with the addition of serum.

Table IV. Lymphoblastic response to *Candida* antigen in different age subgroups

Age groups	*Patients*	*Control*	*p Value*
19-29 yr			
Low response	19 (58%) (n = 33)	4 (17%) (n = 24)	<0.005
30-39 yr			
Low response	15 (48%) (n = 31)	5 (71%) (n = 7)	NS
40-50 yr			
Low response	5 (56%) (n = 9)	4 (67%) (n = 6)	NS

NS, Not significant.

Comment

Recurrent or refractory vaginal moniliasis is an uncommon illness in otherwise healthy women but is associated with protracted discomfort, stress, and frustration in patients. Most family physicians and gynecologists see patients with this condition, but the exact incidence is unknown.

Few studies have addressed the issue of the underlying etiology of this condition. Certainly, conditions with impaired cellular immunity are well-known causes of recurrent moniliasis, such as the acquired immunodeficiency syndrome, immunosuppressive therapy, leukemia, lymphoma, endocrinopathies, chronic mucocutaneous candidiasis, and pregnancy. However, in most of these conditions (except for pregnancy), the moniliasis is not restricted to the involvement of vulvovaginal mucosa. None of the patients studied in our clinic or reported on by others have had extragenital moniliasis.

Studies of the humoral immunity in women with recurrent vaginal moniliasis have not shown any defects.[5] In vaginal candidiasis the antibody response is mainly local, consisting of secretory immunoglobulin A. The pathogenesis of recurrent vulvovaginal moniliasis remains obscure, because the majority of patients have no recognized predisposing factors.[6]

Few studies have investigated the cellular immunity in women with recurrent vaginal moniliasis. Syverson et al.[2] examined the cellular immunity in 9 patients and 29 controls. With skin testing only 33% of patients and 55% of controls showed hypoergy or anergic reaction to *C. albicans.* Thus an impaired cutaneous response to *C. albicans* antigen was not greater in the patients than in the controls. Our findings show a similar percentage of patients (24.7%) with negative reaction to *C. albicans* but a much lower negative reaction in the controls (5.4%).

Syverson et al.[2] also studied lymphocyte transformation and leukocyte migration inhibition. No difference in results was found between patients and controls, but the authors concluded that their study suggests a lack of cellular immune response to *Candida* antigen in women with chronic *Candida* vaginitis. Our study failed

to show any significant defects in lymphoblastic response to *Candida* antigen in the entire patient population, compared with controls. There was also no difference in response to mumps, streptokinase, and phytohemagglutinin between the two groups. However, patients 19 to 29 years old had significantly lower lymphoblastic response to *C. albicans* antigen than age-matched controls. The explanation for this observation is unclear, but it may explain differences between studies and it emphasizes the importance of age-matched controls in future studies.

Witkin et al.[3] reported on the cellular immunity of six patients with recurrent vaginal moniliasis and six controls. No differences were observed in the proliferative responses of lymphocytes to mitogens, but the proliferative response to *C. albicans* was decreased in all patients.

Moreover, serum from patients also suppressed the proliferative response of controls' lymphocytes to *C. albicans*, thus suggesting that a soluble component in sera that blocks the response of peripheral blood lymphocytes to *C. albicans* is produced in these patients. These results are clearly at variance with ours and can only be partly explained. Our study involves a much larger patient population, and it is possible that the patients studied by Witkin et al.[3] fall into the younger age group of 19 to 29 years, where a decrease in proliferative responses of lymphocytes to *C. albicans* occurred in nearly 60% of our patients. However, the ages of the patients studied by Witkin et al.[3] were not provided. Also, the methods used in our study are somewhat different, and so the results may not be directly comparable.

More recently the same group[7] reported on the cellular immunity of a larger population of patients ($N = 65$). Again they found that *Candida*-directed proliferation to *Candida* antigen was reduced at least 70% below the mean control value in 73% of patients. Further analyses on selected patients demonstrated that lymphocytes of patients responded in the normal range to *Candida* if incubated in the presence of control macrophages. It should be noted that even in our patients with low blastogenic response to *Candida* antigen the defect was not often permanent and normal results were obtained on some repeat testing.

Mathur et al.[8] previously reported that lymphocyte responses to concanavalin A were significantly lower than those of matched controls in 26 patients with chronic vaginal moniliasis, whereas lymphocyte response to phytohemagglutinin were normal. Because we did not use concanavalin A in our study and Mathur's group did not test *Candida* and mumps antigen, we are unable to compare the results. The study by Mathur et al.,[8] however, does suggest that there may be a defect in the cytotoxic/suppressor T cells of these patients.

In summary, the cellular immunity of most women with recurrent vaginal moniliasis is normal. A subgroup of women, between 19 and 29 years old, showed a higher incidence of impaired lymphocyte proliferative response to *C. albicans* (58%), and only 25% of all patients demonstrated impaired cutaneous delayed hypersensitivity to *Candida* antigen. Thus in the majority of patients the pathogenesis of the recurrent vaginal moniliasis remains unknown.

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Validity of postnatal assessments of gestational age: A comparison of the method of Ballard et al. and early ultrasonography

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This study investigates the accuracy of the postnatal gestational age assessment method of Ballard et al. to predict very preterm, preterm, term, postterm, and small-for-gestational-age infants, with gestational age by early ultrasonography used as the gold standard. Sensitivity, specificity, and predictive value were examined for each gestational age category, with 4193 single live births occurring during the period from 1982 through 1989 with an ultrasonographic examination done by 20 weeks' gestation and a postnatal assessment at 28 to 44 weeks. These data indicate that the postnatal assessment overestimated the gestational age of preterm births, resulting in an underestimation of very preterm and preterm percentages. Nearly three fourths of the postterm births by ultrasonography were misclassified by the method of Ballard et al. because of underestimation of postterm gestational ages. These data indicate that, for research purposes, appreciable misclassification of preterm, postterm, and small-for-gestational-age infants will occur if gestational age is determined by the technique of Ballard et al. (Am J Obstet Gynecol 1992;166:891-5.)

Key words: Gestational age, prematurity, postterm delivery, intrauterine growth, ultrasonography

Ascertainment of gestational age is essential for the evaluation of newborn risk status. Classically, this determination has been based on the date of last normal menses, if that date was considered reliable. However, the accuracy of this measure has been questioned, and ultrasonography has been proposed as a more precise method for estimating gestation age.[1]

When neither a reliable date of last normal menses (LMP) nor an early ultrasonographic examination is available, clinical postnatal assessments of newborn maturity have been used to estimate the length of pregnancy duration.[2, 3] On the basis of observations of the physical and neurologic characteristics of the newborn, these assessments have been shown to be highly correlated with the LMP interval, have been touted as a valid alternate measure of gestational age,[2-6] and have been frequently used in gestational age–related research.[7]

Lower maternal socioeconomic status has been associated with a missing or uncertain LMP and with late entry into prenatal care, which may preclude an early ultrasonographic examination.[8-12] These same characteristics have further been associated with low birth weight and preterm delivery.[11-15] In these situations, postnatal assessments are of increasing importance in the determination of gestational age. In recent years, however, concern has grown over the inability of postnatal assessments to provide valid estimates of gestational age in low-birth-weight and preterm infants.[16-21] In this study we investigated the ability of a postnatal assessment method to accurately assess the gestational age of very preterm, preterm, term, postterm, and small-for-gestational-age (SGA) infants with gestational age by early ultrasonography used as the gold standard.

Methods

Data were obtained from the Medical University of South Carolina computerized Perinatal Information System.[22] The racially mixed service population of this regional perinatal center is largely composed of lower-income patients. Patient records were prospectively re-

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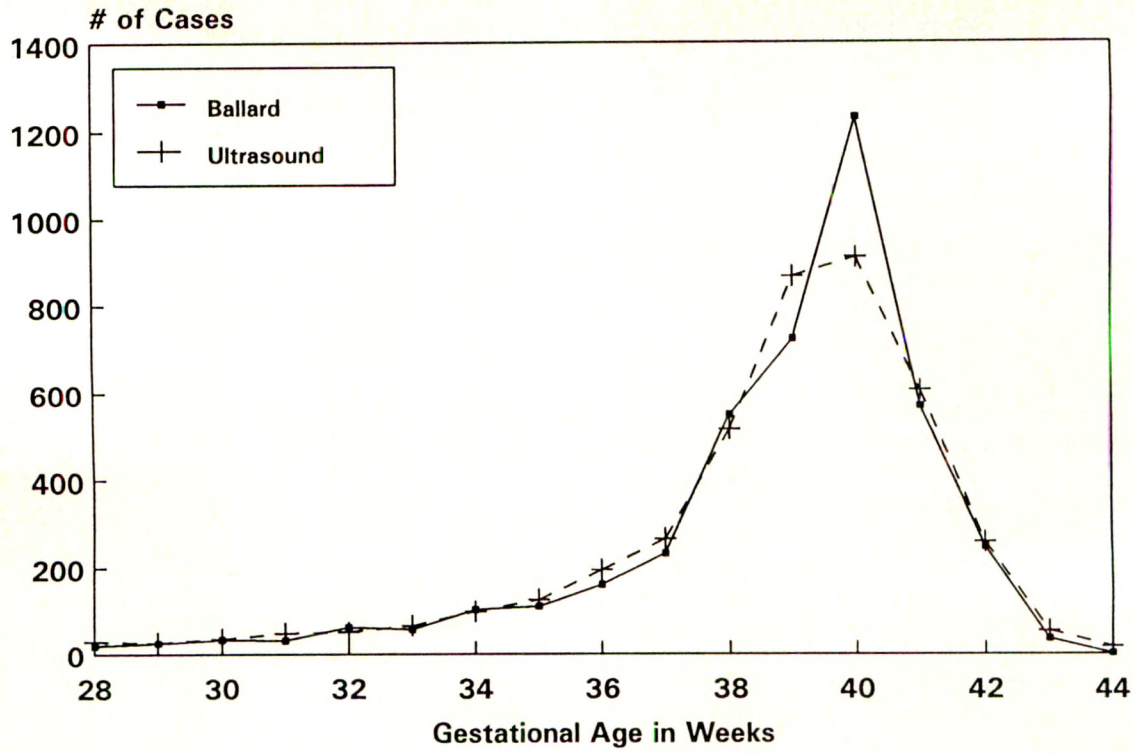


Fig. 1. Gestational age distribution by method of Ballard et al. and ultrasonography.

Table I. Gestational age interval and distribution measures by method by Ballard et al. and early ultrasonography

	Ballard et al.	*Ultrasonography*
Very preterm (<33 wk) (%)	4.05	4.94
Preterm (<37 wk) (%)	14.33	16.53
Term (37-41 wk) (%)	78.89	75.51
Postterm (≥42 wk) (%)	6.77	7.97
SGA (%)	11.34	9.43
Mean ± SD (wk)	38.77 ± 2.58	38.60 ± 2.86
Skewness	−1.61	−1.59
Kurtosis	3.07	3.34

viewed by data clerks with training in medical record abstraction, and a standardized data collection method was used throughout the study period.

Single live births with delivery in the Medical University of South Carolina Hospital during the period from 1982 through 1989 with an ultrasonographic examination by 20 weeks' gestation and a postnatal assessment at 28 to 44 weeks, according to the method proposed by Ballard et al.,[3] were selected for analysis. The Ballard postnatal assessment was routinely performed for all deliveries and was administered by trained clinicians in the course of normal clinical practice. There were 4193 cases that met the study criteria. Infants with conditions that precluded the administration of the Ballard method (e.g., major congenital anomalies) were excluded.

Gestational age by ultrasonography was largely established by a combination of biparietal diameter, femur length, and abdominal circumference.[23] SGA was determined on the basis of the standard provided by Brenner et al.,[24] representing those cases <10th percentile of the birth weight distribution for gestational age.

Results

Although the gestational age distribution by Ballard and ultrasonography exhibit similar mean values (Table I), the Ballard distribution, displayed in Fig. 1, reveals a greater concentration of cases around the 40-week value, with fewer cases in the extreme tails of the distribution. Table I provides, by Ballard and ultrasonography, the percentages of very preterm (<33 weeks), preterm (<37 weeks), term (37-41 weeks), postterm (≥42 weeks), and SGA. The ultrasonography very preterm, preterm and postterm percentages exceed the Ballard percentages, whereas the ultrasonography SGA percentage is lower than that indicated by Ballard. The correlation between the two measures is $r = 0.79$.

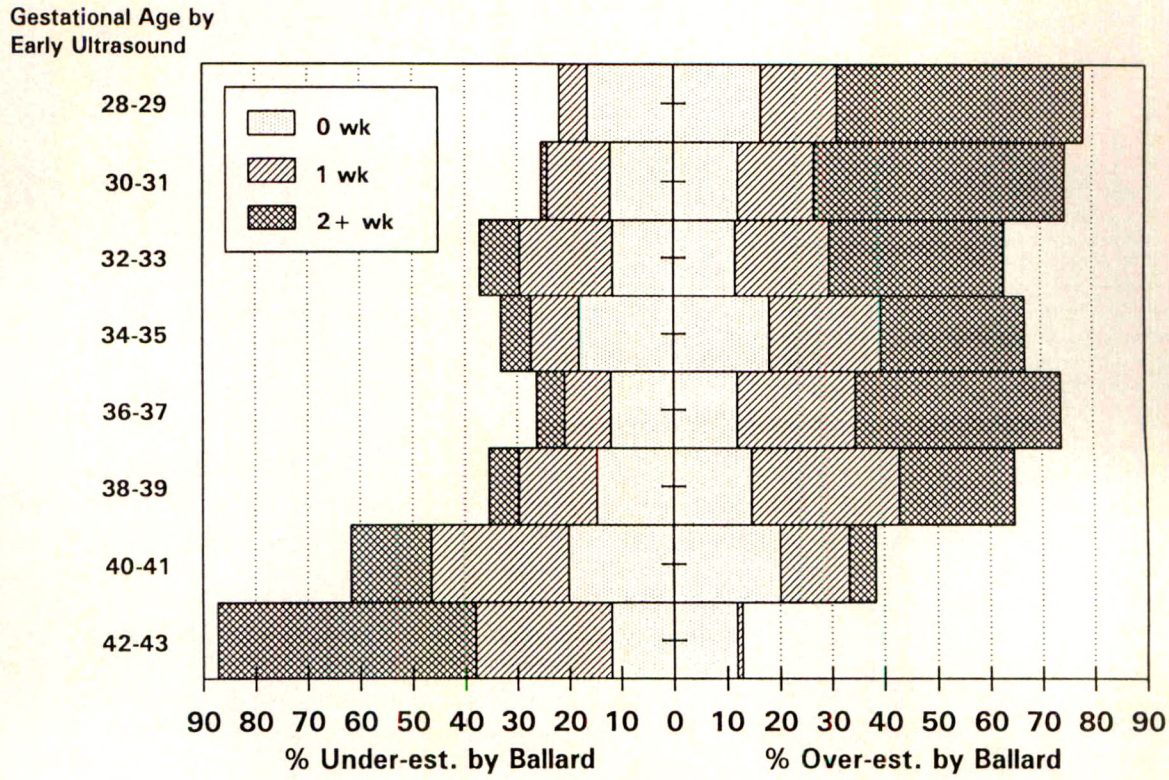


Fig. 2. Percent of cases with early ultrasonography gestational age overestimated and underestimated by Ballard by ≥1 week.

Table II. Efficacy of method by Ballard et al. to identify gestational age–based risk categories as determined by early ultrasonography

	Gestational age–based risk categories				
	Very preterm	*Preterm*	*Term*	*Postterm*	*SGA*
Sensitivity	66.18	72.15	90.93	26.95	76.46
Specificity	99.17	97.11	58.23	94.97	95.44
Positive predictive value	80.59	83.19	87.03	31.69	63.58
Negative predictive value	98.26	94.63	67.57	93.76	97.50

Sensitivity is the percent of cases placed in the category by ultrasonography that were also so identified by Ballard. Specificity is the percent of cases excluded from the category by ultrasonography that were also excluded by Ballard. Positive predictive value is the percent of cases placed in the category by Ballard that were also so identified by ultrasonography. Negative predictive value is the percent of cases excluded from the category by Ballard that were also excluded by ultrasonography.

The percentage of cases with a gestational age by ultrasonography either overestimated or underestimated by Ballard by ≥1 week is shown in Fig. 2. Before 40 weeks there is a decided tendency by Ballard to overestimate the ultrasonographic value. At the interval from 28 to 29 weeks, nearly 50% of the cases were overestimated by Ballard by ≥2 weeks. Among postterm births approximately 50% of the cases were underestimated by Ballard by ≥2 weeks.

Table II reports measures of the efficacy of Ballard to identify the various ultrasonographically determined gestational age groups. Two thirds of the very preterm infants identified by ultrasonography were classified as very preterm by Ballard (sensitivity 66%). For postterm births the sensitivity by Ballard was only 27%. Of those infants designated postterm by Ballard, fewer than one third were postterm by ultrasonography (positive predictive value 32%). Fewer than two thirds of the infants determined SGA by Ballard were SGA by ultrasonography (positive predictive value 64%).

Table III displays the efficacy of Ballard to identify SGA infants as determined by ultrasonography for low birth weight (<2500 gm) and normal birth weight (≥2500 gm) infants. For low-birth-weight infants, approximately two thirds of the infants identified as SGA by Ballard were SGA by ultrasonography. For normal-

Table III. Efficacy of method by Ballard et al. to identify low- and normal-birth-weight infants as SGA with early ultrasonography used as gold standard

	Low birth weight (<2500 gm)	*Normal birth weight (≥2500 gm)*
SGA by Ballard (%)	46.06	5.00
SGA by ultrasonography (%)	38.18	4.18
Sensitivity	80.57	69.59
Specificity	75.25	97.82
Positive predictive value	66.78	58.19
Negative predictive value	86.25	98.66

Sensitivity is the percent of cases placed in the category by ultrasonography that were also so identified by Ballard. Specificity is the percent of cases excluded from the category by ultrasonography that were also excluded by Ballard. Positive predictive value is the percent of cases placed in the category by Ballard that were also so identified by ultrasonography. Negative predictive value is the percent of cases excluded from the category by Ballard that were also excluded by ultrasonography.

birth-weight infants, the positive predictive value of Ballard was even lower (58%).

Ultlrasonographic technology was changing over this period and showed an increased use during the latter years of the study period. An examination of the data by year did not reveal a temporal trend in these findings.

Comment

The greater central concentration of the Ballard distribution suggests a tendency by Ballard examiners to err toward the mean. For preterm births the postnatal assessment overestimated the early ultrasonography-based gestational age, resulting in an underestimation of very preterm and preterm percentages. In addition, nearly three fourths of the postterm births by ultrasonography were misclassified by Ballard because of underestimation of postterm gestational ages. Because of these systematic errors, the conventional assumption that postnatal assessments have a random ±2-week error margin appears faulty for nonterm births. Agreeing with previous findings,[16-21] these results raise serious questions regarding the ability of the Ballard technique to accurately identify either preterm or postterm infants.

It has been previously reported that the Ballard method underestimates the gestational age of SGA births compared with non-SGA births.[6] This was also found in this study (data not shown). The overestimation by Ballard of the gestational age of non-SGA births results for some cases in the application of higher-birth-weight criteria to establish SGA. This leads to a misassignment of some of these births to the Ballard SGA category and inflates the overall Ballard percentage of SGA births.

These data indicate that, for research purposes, appreciable misclassification of preterm, postterm, and SGA infants will occur if gestational age is determined by Ballard. It further suggests that given a case series for which some of the cases are lacking gestational age information from a certain LMP or an early ultrasonographic examination, it is unwise to substitute a gestational age value derived from a postnatal assessment. As an uncertain LMP or missing early ultrasonography is more likely to occur among cases with lower socioeconomic characteristics, as determined by maternal age, race, education, marital status, or payment method, this practice may produce biased gestational age estimates for higher-risk subgroups, thereby confounding the research results.

The initial postnatal assessment validation studies were conducted with a limited number of examiners with an emphasis on reliability and internal validity issues.[2, 3] The Ballard scores in this investigation were taken under the more normal practice conditions found in a large university hospital by numerous trained recorders who were unaware of this research agenda. As such, the quality of these postnatal assessment data may more closely reflect that typically found on large perinatal data bases and what is more commonly available for research purposes as a measure of gestational age. Although more controlled and limited investigations may improve on these results, these data have implications for the accuracy of postnatal assessment methods used in more broad and representative situations and for the many studies that use postnatal assessment estimates obtained in a similar manner.

Although high correlations between postnatal assessments and gestational age from LMP have been demonstrated, the adequacy of correlation techniques to establish the validity of postnatal assessments has been questioned.[19, 25] In these data we find that the postnatal assessment was most sensitive in identifying births that were determined to be at term by ultrasonography. Unfortunately, a postnatal assessment is most useful if it can also provide an accurate assessment of nonterm infants. As evidence mounts concerning the inability of these methods to accurately identify infants

in more at-risk gestational categories, future research should focus on modifying or reformulating current postnatal assessment criteria.

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Diagnosis of cervical change in pregnancy by means of transvaginal ultrasonography

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There are no absolute objective diagnostic criteria for cervical incompetence. Abdominal and endovaginal ultrasonographic assessment of endocervical length, both of which have been associated with technical problems, have been used to establish the diagnosis. Cervical cerclage may be useful in preventing silent cervical dilatation; however, no prospective trials with and without cerclage have been performed when endocervical shortening has been demonstrated by ultrasonography. (AM J OBSTET GYNECOL 1992;166:896-900.)

Key words: Cervical incompetence, endovaginal ultrasonography, cervical cerclage

Diagnosing cervical incompetence is difficult because there are no specific tests or criteria that confirm or exclude the diagnosis.[1] At our institution, in April, 1990, Kushnir et al.[2] reported on the use of transvaginal ultrasonography to determine the normal length of the endocervical canal during gestation. We use this technique in the routine monitoring of patients at risk for cervical incompetence. The following summaries describe two cases in which transvaginal ultrasonographic assessment of the cervix resulted in the decision to place a cerclage.

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6/1/33024

Case reports

Case 1. The patient, a gravida 4, para 0, black woman, had a history of two first-trimester spontaneous abortions and a pregnancy loss at 22 weeks. She reported no perception of labor before spontaneous rupture of membranes and subsequent rapid delivery of a stillborn infant. Other pertinent history included chronic hypertension, smoking one pack of cigarettes

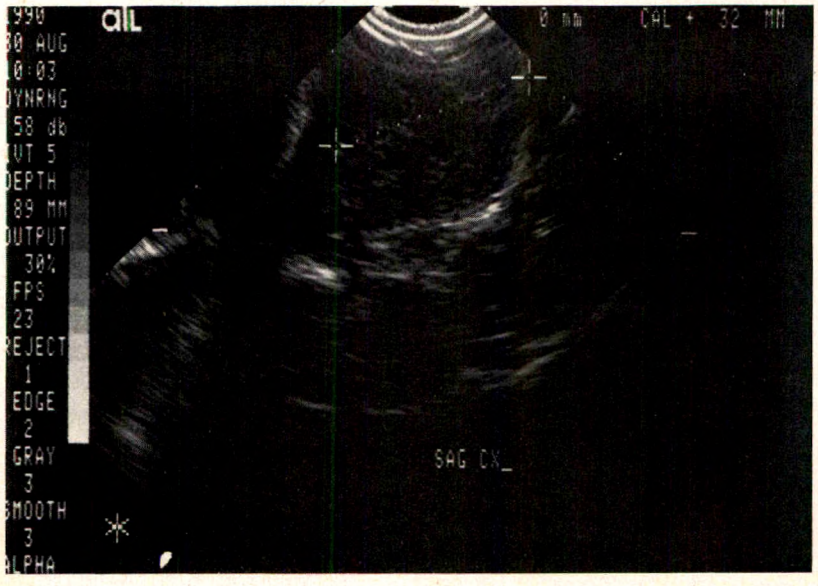


Fig. 1. Endovaginal ultrasonographic measurement indicates that endocervical length of patient in case 1 has decreased to 32 mm at 25 weeks' gestation.

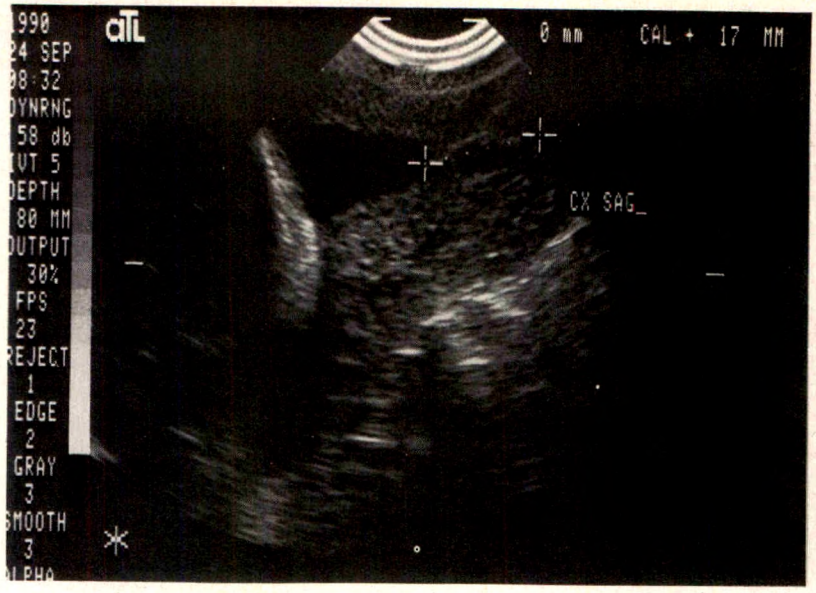


Fig. 2. At 28 weeks of gestation there is marked beaking of chorioamnion with internal os dilatation. Length of remaining endocervix is 17 mm.

per day, and consuming three to five bottles of beer per week. She presented to the Maternal-Fetal Medicine clinic at our institution at 18 weeks' estimated gestational age. A physical examination revealed bacterial vaginosis, which was subsequently treated with metronidazole (Flagyl, Searle & Co., Chicago). In results of the digital examination, the cervix was described as "long, thick, and closed." Vaginal ultrasonographic assessment revealed an endocervical length of 55 mm. Estimated gestational age indicated by transabdominal ultrasonography at this time was 18 weeks 6 days.

To determine the patient's endocervical length, serial ultrasonographic examinations were performed with an ADR Ultramark IV scanner (ADR, Bothel, Wash.) with either a 3.5 or 5.0 MHz transvaginal transducer. The endocervical length decreased from 63 to 32 mm between 22 weeks and 25 weeks of gestation. At 28 weeks marked dilatation of the internal os with "beaking" of chorioamnion was visible. The remaining endocervix measured 17 mm in length (Figs. 1, 2, and 3).

The patient was evaluated for preterm labor by means of external tocodynamometry, but no contractions were noted. The extent of cervical shortening was not evident on digital examination. A cerclage was placed at 29 weeks' gestation. At surgery, the cervix appeared closed and the membranes were not visible, in spite of marked dilatation of the internal os seen on transvaginal ultrasonography. The pregnancy was further complicated by mild preeclampsia and chronic hypertension.

On Nov. 8, 1990, at 35 weeks' estimated gestational age, the patient experienced spontaneous rupture of membranes and the cerclage was removed. She was delivered of a female infant who weighed 1930 gm with Apgar scores of 9 at both 1 and 5 minutes. The infant went home with her mother on postpartum day 2.

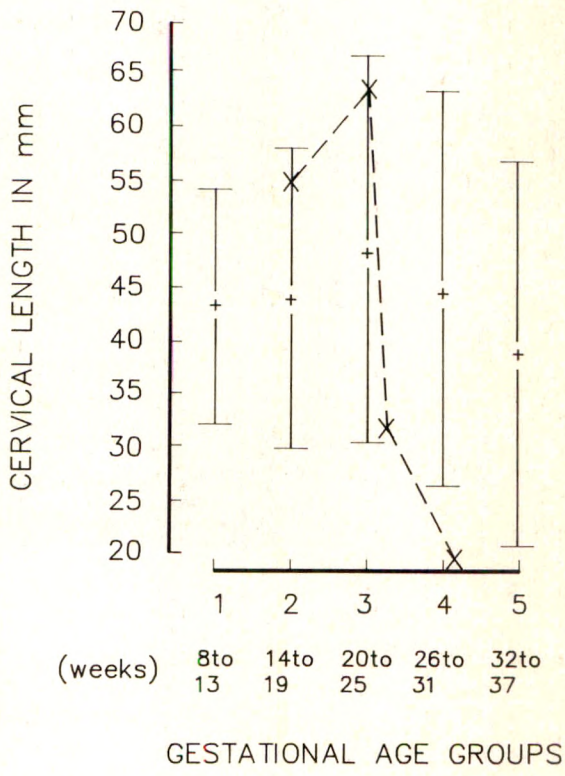


Fig. 3. *Plus sign,* Median (±2 SD) for endocervical length at various stages of gestation as reported by Kushnir et al.[2] ×, Measurement of endocervical length of patient in case 1 at 18, 21, 25, and 28 weeks of gestation.

Case 2. The patient, a 24-year-old, gravida 3, para 0 white woman, first presented to the Maternal-Fetal Medicine clinic at 10 weeks of gestation. She reported a history of two first-trimester spontaneous abortions

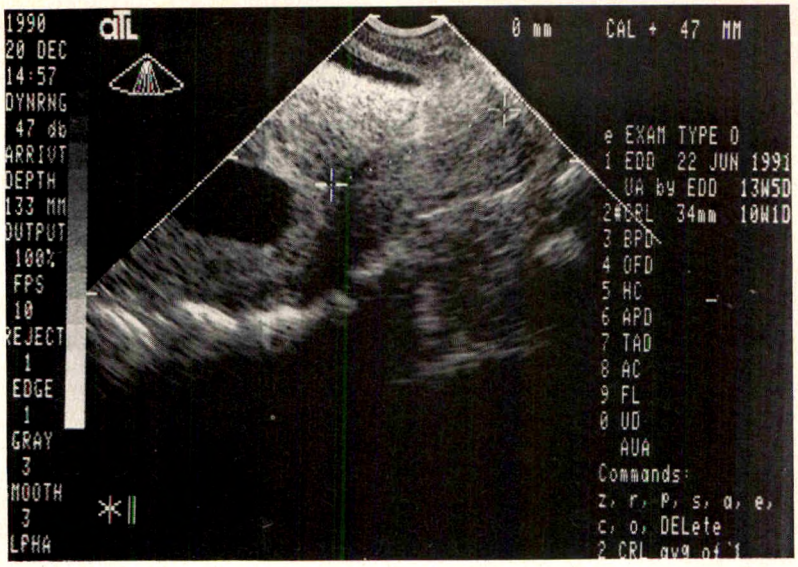


Fig. 4. Endocervical length of patient in case 2 at 10 weeks of gestation. Note prominent endometrial stripe.

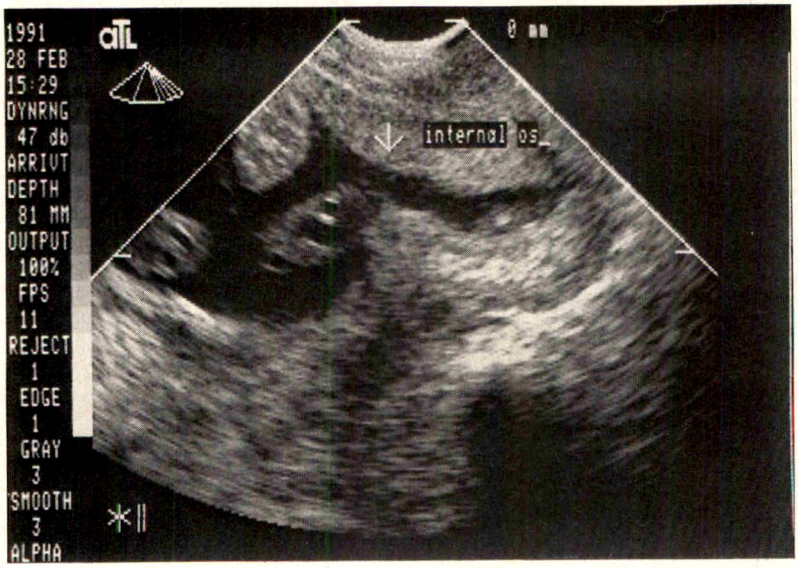


Fig. 5. Same patient as in Fig. 4 at 19.5 weeks' gestation. Note beaking of amniotic fluid past internal os of cervix. Also note section of umbilical cord presenting at internal os. Endocervical length was measured as 27 mm.

followed by curettage and a pregnancy loss at 24 weeks' gestation. Her history was otherwise unremarkable, and she had had no known in utero exposure to diethylstilbestrol.

Endovaginal ultrasonographic assessment of the cervix was completed in a serial fashion. At 10 weeks' estimated gestational age, the endocervical length measured 47 mm (Fig. 4). At 15 weeks the endocervical length was reportedly 37 mm. At 19.5 weeks the endocervical length had diminished to 27 mm, and there was marked beaking of the chorioamnion into the endocervical canal (Fig. 5). The decision was made to place a cerclage. Speculum examination of the cervix at the time of surgery revealed that the bag of waters was visible at the cervical os. The bladder was filled with 300 ml of sterile water to assist in retracting the bag of waters from the cervix. Cerclage placement was otherwise uneventful.

The patient returned for an ultrasonographic examination to determine interval growth at 25 weeks'

gestation. The amniotic fluid index was 7.2 cm. Oligohydramnios as a result of rupture of membranes at cerclage placement was strongly suspected, although the patient denied fluid leakage. Amniocentesis with dye instillation was performed, and rupture of membranes was not documented. Five days later the repeat amniotic fluid index was 14.2 cm.

The patient presented again at 33 weeks' gestation with complaints of uterine contractions and vaginal bleeding. Intravenous tocolysis was initiated, and amniocentesis was performed to rule out intraamniotic infection and to document lung maturity. Amniotic fluid Gram's stain and cultures were negative. The lecithin/sphingomyelin ratio was 6.7 with phosphatidylglycerol present. The estimated fetal weight was 1800 gm. The decision was made to remove the cerclage because the patient continued to labor in spite of tocolysis. The patient was quickly delivered of an 1810 gm female infant with Apgar scores of 7 and 9 at 1 and 5 minutes, respectively. Inspection of the placenta revealed an adherent 3 cm clot consistent with chronic abruptio placentae.

Comment

The diagnosis of cervical incompetence relies on the demonstration of cervical dilatation in the absence of uterine contractions. However, as demonstrated in case 1, significant and dangerous shortening of the endocervix may occur before dilatation of the cervix can be demonstrated by speculum examination or digital palpation. Thus diagnostic methods such as transvaginal ultrasonography, which more objectively demonstrate earlier cervical changes, become very attractive.

In addition, a significant percentage of primigravid and multiparous women have dilatation of 1 to 2 cm at 21 to 28 weeks' gestation, and they do not have a higher rate of preterm delivery. With the development of nomograms of endocervical length in large population studies, identification of this group of patients with "physiologic" cervical dilatation who are at low risk for preterm delivery may some day be accomplished.

Ayers et al.[3] established a nomogram for cervical length in a group of 142 women who were subsequently delivered at term. According to Ayers et al., "Cervical length observed . . . was remarkably constant, averaging 52 mm until effacement began at 33-34 weeks gestation." Both Kushnir et al.[2] and Ayers et al.[3] have demonstrated no significant difference in cervical length between primigravid and multigravid women who were eventually delivered at term. However, in a study by Kushnir et al.,[2] in contrast to that by Ayers et al.,[3] a shorter mean cervical length at all gestational ages and a significant increase in cervical length between 20 and 25 weeks' gestation were noted.

The differences noted (a shorter cervical length and variability in length with increasing gestational age) might result from the greater diagnostic accuracy of the vaginal probe. As Kushnir et al.[2] stated, "Cervical distortion is avoided because the vaginal probe is in close proximity to the cervix."

The demonstration of beaking of membranes with internal os dilatation probably represents the final stage of endocervical shortening before frank prolapse past the external os. In our experience serial scans have always demonstrated persistent endocervical shortening before beaking of the membranes.

When either transvaginal or abdominal ultrasonography is used to evaluate endocervical length, several measurements should be repeated during the session. Transient contraction of the lower uterine segment can lead to false elongation of endocervical length and can give the false image of beaking of the membranes. As a result of the obstetric histories of the patients described in this review, many clinicians would have opted for immediate placement of cerclage. Placement of cerclage has become the standard of care for these patients, in whom other causes of recurrent midtrimester pregnancy loss, such as uterine anomaly or infection, have been eliminated. However, significant data on which to make the decision to place cerclage are lacking. Most reports of successful pregnancy outcome after placement of cerclage use the patients' earlier pregnancy outcomes for comparison. These comparisons are not valid, because several series report that as many as 70% of patients who have a mid-trimester loss will be delivered at term with no treatment in their subsequent pregnancies. Because placement of cerclage has potential risks even in the second trimester, reserving the procedure for those patients who have endocervical shortening demonstrated on transvaginal scan might be prudent. The evaluation of endocervical length may also be valuable in the patient with a history suggestive of cervical incompetence, but who presents for her first prenatal care after 20 weeks' gestation.

The question of whether to place a cerclage in the late second or early third trimester after the demonstration of significant shortening in cervical length remains difficult. In the study of Ayers et al.[3] 97% of patients who received a cerclage after demonstration of cervical shortening were delivered after 35 weeks' gestation. However, to our knowledge there are no prospective studies comparing the rate of preterm delivery after demonstrated endocervical shortening with and without cerclage. In case 1 we thought that the risk of preterm delivery outweighed the hazards involved in the placement of cerclage. However, that patient may have had the same outcome with bed rest alone.

In case 2 the patient had at least two of the problems

encountered when a cerclage is placed in the late second trimester and after dilatation of the internal os: possible preterm rupture of the membranes and preterm labor.

In summary, vaginal ultrasonography may provide a more objective basis for the diagnosis and management of cervical incompetence. A prospective study is needed to evaluate the risks and benefits of cerclage in the prevention of preterm delivery when cervical shortening has been demonstrated by vaginal ultrasonographic assessment.

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Contraceptive compliance with a levonorgestrel triphasic and a norethindrone monophasic oral contraceptive in adolescent patients

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OBJECTIVE: This study was undertaken to assess the impact of two low-dose oral contraceptive pills on compliance and side effects in adolescent patients.

STUDY DESIGN: The use of a levonorgestrel-containing triphasic pill ($N = 114$) was compared with that of a monophasic (1 + 35) norethindrone-containing pill ($N = 110$) at two different sociodemographic sites.

RESULTS: No significant difference in compliance or pill satisfaction was observed between the pills. Socioeconomic factors were the overriding predictors of compliance. At 3 and 12 months of follow-up, there were significantly fewer compliants of overall side effects ($p < 0.001$ and $p = 0.004$, respectively), breakthrough bleeding ($p = 0.017$ and $p = 0.018$), and pill amenorrhea ($p = 0.002$ and $p < 0.001$) among users of the triphasic pill. Mean weight change at 12 months was +1.1 kg for the monophasic pill and −0.1 kg for the triphasic pill. All known pregnancies occurred among noncompliant city clinic patients.

CONCLUSIONS: Adolescents experienced fewer side effects with the triphasic pill than with the monophasic one, but compliance was the same. (Am J Obstet Gynecol 1992;166:901-7.)

Key words: Contraceptive compliance, adolescents, birth control, oral contraceptive

Adolescent pregnancy continues to be a major problem in the United States, with rates considerably higher than those of other developed countries.[1, 2] Initiation of contraception continues to be delayed and inconsistent.[1-11] In a previous study in our clinics,[3] many of the predictors of compliance in adolescents with follow-up at three different sites (an inner-city adolescent clinic, a birth control clinic in a midsized industrial city, and a suburban private practice) were sociodemographic factors that are difficult for health care professionals to change. However, the lack of real or perceived side effects was associated with improved compliance.

The new triphasic oral contraceptive pills have lower progestin and estrogen content than many commonly prescribed monophasic pills. However, it is unclear if adolescents can follow the more complex packaging of the triphasic pills. This study was undertaken to assess the impact on reported side effects and adolescent compliance with a levonorgestrel-containing triphasic pill compared with our previous study of a norethindrone-containing (1 + 35) monophasic pill.[3]

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6/1/33390

Material and methods

Two hundred twenty-four patients who were starting oral contraceptives for birth control for the first time were entered into the study. Patients were recruited from two different sites: a hospital-based city clinic ($N = 124$) and a suburban adolescent private practice ($N = 100$) with 114 receiving a levonorgestrel-containing triphasic pill (Triphasil 28d, Wyeth-Ayerst Laboratories, Philadelphia) and 110 receiving a monophasic norethindrone-containing pill (Norinyl 1 + 35, Syntex Laboratories, Palo Alto, Calif.). In compliance with our Human Investigation Committee's guidelines for quality improvement, standard medical care was given and informed consent for initiating oral contraceptive pills was obtained.

As in our previous study, which included only patients using a monophasic pill,[3] data about demographics, sexual history, previous birth control use, career goals, concerns about potential side effects, and parental and partner knowledge of the contraceptive pill were collected. A physical examination including height, weight, blood pressure, and pelvic examination was performed. Each patient was provided with a 3-month supply of contraceptive pills. Patients were given

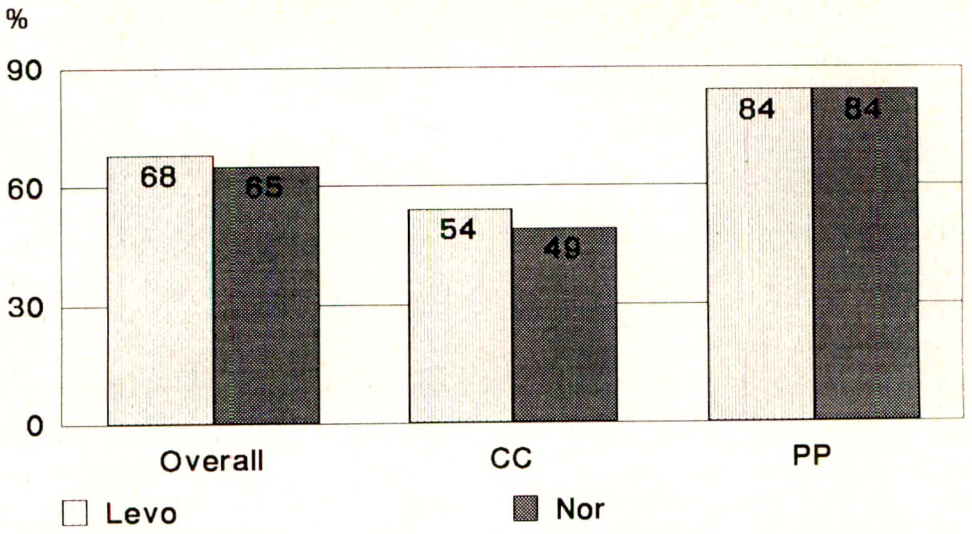


Fig. 1. Compliance with levonorgestrel triphasic *(Levo)* and norethindrone monophasic *(Nor)* oral contraceptive at 3 months of follow-up. *CC*, City clinic patients; *PP*, private practice patients.

Table I. Demographics of patients receiving levonorgestrel-containing pill compared with norethindrone-containing pill

	Levonorgestrel-containing pill (n = 114)	*Norethindrone-containing pill (n = 110)*	*p Value*
Site of health care			
Adolescent clinic	63	61	0.98
Private practice	51	49	
Residence			
Metropolitan	47	56	0.29
Suburban	47	35	
School	20	19	
Race			
Black	36	43	0.23
White	61	72	
Insurance			
Medicaid	21	26	0.34
Other	93	84	
Age (yr, mean ± SD)	17.4 ± 2.3	17.0 ± 2.0	0.15

the monophasic pill in the first block of time (December 1983 through June 1984) and the triphasic pill in the subsequent block of time (May 1985 through June 1987).

Follow-up information was obtained at the 3-month visit and at the visit closest to 12 months for long-term follow-up. Data at follow-up visits included blood pressure, weight, menstrual pattern, side effects, pregnancy, and compliance with taking the oral contraceptives. Compliance was defined as both returning for the follow-up visit and taking the pill as prescribed or stopping the pill because of no longer needing contraception.

Data analysis was performed with the SAS statistical package.[12] Fisher's exact test (two-tailed) or χ^2 test was used for all categoric variables. For continuous variables the appropriate *t* test was performed when comparison groups had equal variances (as assessed by the *F*-test result), and an approximation of the *t* test was performed when comparison groups had unequal variances.[13] All results are reported as mean ± SD. SAS's general linear model, stepwise regression, and CATMOD logistic regression subroutines were used to assess the predictor's relative contribution to R^2 and the overall significance of the models.

Results

The mean age of the 224 patients was 17.2 ± 2.1 years; 21% received Medicaid; 37% were black and 63% white; 46% resided in the city, 37% in the suburbs, and 17% at school. There were no significant demographic differences between patients started on a regimen of

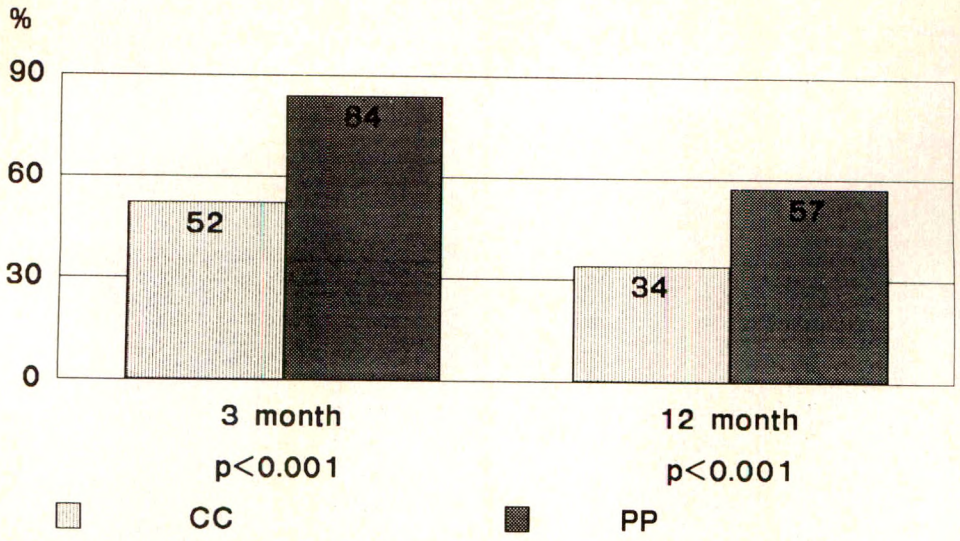


Fig. 2. Compliance at private practice *(PP)* and city clinic *(CC)* of all patients initiating either study oral contraceptive.

monophasic or triphasic pills (Table I). At 3 months of follow-up 181 patients (81%) returned, and 148 (66%) were classified as compliant with 132 (59%) still taking the pill. There was no significant difference in compliance as a result of the type of pill either for the total group or when stratified by primary care site (Fig. 1), but compliance was significantly different when city clinic patients were compared with private practice patients (Fig. 2). Overall, compliant patients were more likely to use contraception before starting the pill, to express more satisfaction with the pill, and to have fewer real and perceived side effects with the pill than were noncompliant patients (Table II).

At 12 months of follow-up (mean 12.7 ± 3.9), 141 (63%) patients returned for a visit, and only 99 (44%) patients were still classified as compliant, with 66 (29%) taking the pill. No significant difference in compliance was attributed to the type of pill in the total sample or when stratified by primary care site (Fig. 3), but compliance was again significantly different between the clinic sites (Fig. 2). Patients compliant at 12 months were older at entry (18.2 ± 2.2 vs 17.4 ± 2.0 years, $p = 0.0021$) than noncompliant patients. Compliant patients were less likely to be concerned about possible pregnancy, more satisfied with the pill, and less likely to experience nausea with the pill (Table III).

The demographic variables associated with compliance in the overall group were the same for the triphasic pill group alone at 3 and 12 months, except that older age ($p = 0.088$) and father's level of education ($p = 0.32$) did not reach the level of significance at 3 months of follow-up. Compliant triphasic patients at 3 months had the same significant associations as the overall group, except a marginally significant association with prior use of contraception ($p = 0.076$), lack of headache ($p = 0.069$), and lack of nausea ($p = 0.085$). At 12 months of follow-up, prior use of birth control ($p = 0.0027$) was significantly associated with compliance for triphasic patients.

There were significantly more complaints of overall side effects, breakthrough bleeding, and amenorrhea at 3 and 12 months of follow-up for the monophasic contraceptive pill compared with the triphasic pill (Table IV). Weight change for the two oral contraceptive pills was the same at 3 months; a marginally significant difference was noted at 12 months (Table V). No significant difference in pregnancy rates was noted between the triphasic and monophasic pill at 3 months (3 [3%] vs 2 [2%]; $p = 1.0$) or at 12 months (7 [6%] vs 9 [8%]; $p = 0.46$). All known pregnancies were among city clinic patients who stated that they had stopped the pills. At long-term follow-up, the overall pregnancy rate was 8.2 pregnancies per 100 person years, the rate for city clinic patients was 19.2 pregnancies per 100 person years, and the rate for noncompliant city clinic patients was 49.6 pregnancies per 100 person years.

Logistic regression analyses identified the city clinic site of initial visit as the strongest predictor of oral contraceptive noncompliance at 3 months ($p = 0.001$) and 12 months ($p = 0.0045$) of follow-up. Pill type had no significant impact on compliance. At 3 months of follow-up, overall side effects ($p = 0.012$) and breakthrough bleeding ($p = 0.0065$) were significant predictors of noncompliance. When all side effects were modeled together at 3 months, nausea ($p = 0.015$) and perceived weight gain ($p = 0.022$) were the only significant predictors. At 12 months of follow-up, only

Table II. Variables associated with oral contraceptive compliance at 3 months

	No. compliant	*Compliant (%)*	*p Value*
Initial visit variables			
Site			
Adolescent clinic	64	52	<0.001
Private practice	84	84	
Residence			
Metropolitan	54	52	<0.001
Suburban	59	72	
School	35	90	
Race			
Black	37	47	<0.001
White	105	79	
Payment			
Medicaid	24	51	0.015
Other	124	70	
Father's level of education			
High school graduate or less	34	51	0.002
Some college	12	75	
College graduate	50	85	
Graduate school studies	22	65	
Prior use of contraception			
Always	45	76	0.005
Sometimes	45	70	
Never	40	51	
3 mo visit variables			
Side effects of pill use at 3 mo visit			
Perceived weight gain			
Yes	11	55	0.001
No	135	85	
Nausea			
Yes	7	50	0.002
No	139	84	
Headaches			
Yes	3	33	0.001
No	143	84	
Mood changes			
Yes	3	43	0.023
No	143	83	
Worry about being pregnant since pill prescription			
Yes	11	46	<0.001
No	124	87	
Satisfied with pill			
Yes	126	93	<0.001
Somewhat	15	56	

nausea ($p = 0.0081$) was a significant predictor of noncompliance.

Comment

Many of the factors that have an impact on adolescents' compliance with oral contraceptives are sociodemographic and are associated with poverty and lack of life opportunities, which leads to a sense of low self-esteem and hopelessness.[3-11, 14] No significant change in compliance or in overall pill satisfaction between the triphasic and monophasic pills was noted. However, adolescents on the triphasic pill experienced fewer side effects, breakthrough bleeding, and pill amenorrhea. Whereas several real or perceived side effects did have an impact on compliance in the total population in this study, the differences in side effects as a result of the two different pills were not reflected in an overall difference in compliance in spite of the large numbers in this study with sufficient power to pick up a moderately small effect. Any impact of the reduced side effects was too small to detect or was counterbalanced by the effect of packaging or some other minor effect not measured.

Although one weakness of this comparison is that patients were recruited in two different time intervals, fortunately no intervening new publicity about oral contraceptives appeared in the lay press. In addition, there were no significant differences in initial demographics between the two groups, and the same demographic and follow-up variables were consistent predictors of compliance for the triphasic pill alone, as well as for the monophasic group.[3]

Anticipatory guidance should be targeted to the patient population. Adolescent girls are particularly concerned about weight gain[3] and should be told about the findings of no significant mean weight change with either contraceptive studied. Perception of weight gain can lead to discontinuance of oral contraceptives, and thus any concern should be followed by office mea-

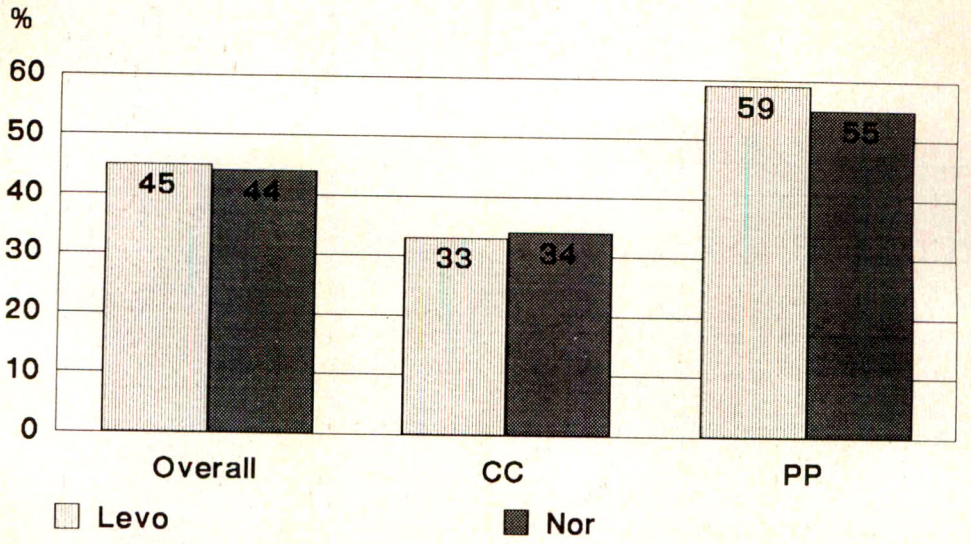


Fig. 3. Compliance of levonorgestrel triphasic *(Levo)* and norethindrone monophasic *(Nor)* oral contraceptive at 12 months of follow-up. *CC*, City clinic patients; *PP*, private practice patients.

Table III. Variables associated with long-term oral contraceptive compliance

	No. of compliants	*Compliant (%)*	*p Value*
Initial visit variables			
Site of health care			
Adolescent clinic	42	34	0.001
Private practice	57	57	
Residence			
Metropolitan	35	34	0.003
Suburban	48	58	
School	16	41	
Race			
Black	25	32	0.003
White	70	53	
Insurance			
Medicaid	12	26	0.004
Other	49	88	
Mother's level of education			
High school graduate or less	39	35	0.001
Some college	8	29	
College graduate	36	67	
Graduate school studies	12	57	
Father's level of education			
High school graduate or less	19	28	0.006
Some college	7	44	
College graduate	35	59	
Graduate school studies	19	56	
Long-term visit variables			
Side effects of pill use			
Nausea			
Yes	4	36	0.007
No	95	77	
Worry about being pregnant since pill prescription			
Yes	8	24	<0.001
No	91	86	
Satisfied with pill			
Yes	84	90	<0.001
Somewhat	7	37	

surements of weight. During the provider's introduction of an oral contraceptive, reassurance and discussion of the management of breakthough bleeding and pill amenorrhea are essential. Nausea and other side effects require prompt attention to prevent the adolescent from discontinuing use. Verbal and written instructions are crucial.[3]

In adolescent patients at either site the poor long-term compliance is of concern. Adolescents who are at high risk for pregnancy must be seen at more frequent

Table IV. Side effects at 3 and 12 months

Total	*Levonorgestrel-containing pill*		*Norethindrone-containing pill*		*p Value*
	No.	*%*	*No.*	*%*	
3 mo					
Total No.	92		89		
Overall	34	37	59	67	<0.001
Breakthrough bleeding	22	24	36	41	0.017
Pill amenorrhea	1	1	11	12	0.002
12 mo					
Total No.	70		71		
Overall	17	24	31	48	0.004
Breakthrough bleeding	6	9	15	23	0.018
Pill amenorrhea	0	0	11	17	<0.001

Table V. Weight change

	Pill	*Weight change (kg, mean ± SD)*	*p Value*
3 mo	Levonorgesterel-containing pill	+0.4 ± 2.2	0.81
	Norethindrone-containing pill	+0.5 ± 2.3	
	Overall	+0.5 ± 2.3	
12 mo	Levonorgesterel-containing pill	−0.1 ± 4.0	0.058
	Norethindrone-containing pill	+1.1 ± 3.3	
	Overall	+0.5 ± 3.7	

intervals than adults. Reaching out to at-risk teens with school problems, parenting sibs, sexually transmitted diseases, and substance abuse is critical. As in our previous study,[3] all known pregnancies were among noncompliant city clinic patients. The lack of pregnancies among private practice patients likely reflects the greater use of backup methods and motivation to avoid pregnancy even when the oral contraceptive has been discontinued.[3] It is probable that additional adolescents in both groups missed pills during cycles but did not become pregnant. Self-report of overall compliance in taking the pill or stopping because of not needing contraception is at least a sufficient marker of efficacy because none of these compliant adolescents became pregnant.[3, 6] Adolescents may have continued to take oral contraceptives prescribed in other settings, and thus compliance may have been underestimated. Anticipatory guidance at the initial gynecologic visit should always include discussions of condoms for backup and for reducing exposure to sexually transmitted diseases.

Adolescent pregnancy and compliance with birth control methods continue to be a major issue for providers and society. Confidential services must be provided with careful discussions of the safety and side effects of oral contraceptives to optimize adolescents' compliance. Beyond what physicians can do in their offices, improvements are needed in the areas of governmental policy, welfare, job and educational opportunities, school-based clinics, preventative education, and peer counseling to reduce the impact of the sociodemographic inequalities and internal sense of hopelessness that contribute to adolescent pregnancy.

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Prenatal ultrasonographic diagnosis of fetal scrotal inguinal hernia

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Fetal scrotal inguinal hernia was diagnosed at 33 weeks of pregnancy by ultrasonographic examination. Moving, echo-free, cystlike structures representing peristalsis within trapped loops of bowel in an abnormally enlarged scrotum were the main ultrasonographic finding. The diagnosis was confirmed post partum, and surgery was successfully carried out 4 days after delivery. (AM J OBSTET GYNECOL 1992;166:907-9.)

Key words: Prenatal diagnosis, ultrasonography, inguinal hernia

The most common defect in the abdominal wall in infants and children is indirect inguinal hernia.[1] The early detection of this pathologic condition is extremely important because segments of bowel may become trapped in the hernia pouch, subsequently leading to partial or complete obstruction of the lumen. We report a case of scrotal inguinal hernia diagnosed at 33 weeks of gestation. To the best of our knowledge this is the first report in the English literature of in utero diagnosis of fetal inguinal hernia.

Case report

A 21-year-old-woman, gravida 0 para 0, was referred for ultrasonography at 33 weeks' gestation because the fundal height did not correspond to the dates. On ultrasonographic examination, a single vertex-presenting fetus was observed. Measurements of biparietal diameter, head circumference, abdominal circumference, and femur length (86 mm, 30.6 cm, 27.5 cm, and 65 mm, respectively) corresponded perfectly to the dates. No other fetal abnormalities were detected. The fetus was male, and a huge scrotum measuring 65 × 56 × 64 mm was observed (Fig. 1). The penis was pushed aside by the enlarged scrotum. The testicles were very close to each other. Small, fluid-filled, cystlike formations occupied a considerable part of the scrotum. These structures demonstrated obvious peristalsis and represented loops of small bowel trapped in the scrotum (Fig. 2).

On the basis of this observation scrotal inguinal hernia was diagnosed. No intraabdominal pathologic condition that would cause elevated pressure within the abdominal cavity was observed on repeated ultrasonographic examinations performed at 2-week intervals. No evidence of bowel obstruction was found in the repeated scans, and the patient was allowed to continue the pregnancy. Normal spontaneous delivery occurred at term, and a male infant weighing 3.4 kg was delivered vaginally.

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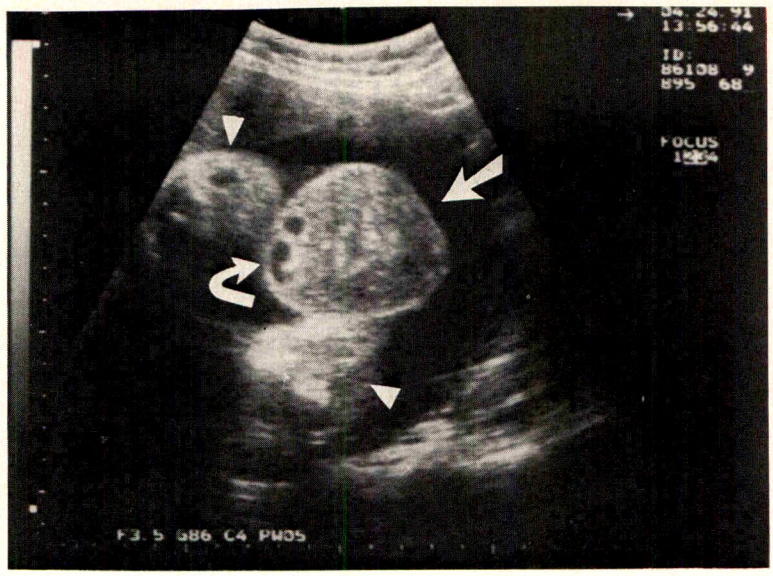


Fig. 1. Ultrasonographic scan at 36 weeks' gestation. Note huge scrotum *(arrow)* as compared with both thighs *(arrowheads)*. Loops of bowel are evident inside scrotum *(curved arrow)*.

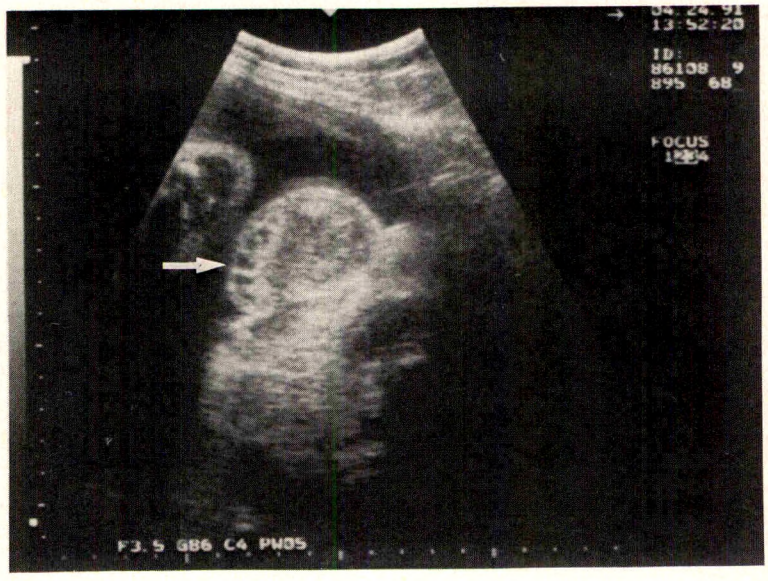


Fig. 2. Ultrasonogram illustrating trapped loop of bowel undergoing wave of peristalsis inside scrotum. *Arrow*, Loop of bowel.

The infant appeared normal except for a huge scrotum that was opaque to transmitted light (Fig. 3). Marked swelling was noted at the left external inguinal ring extending downward into the scrotum. On examination both testicles were palpated. A scrotal mass representing condensed bowel loops was also encountered. Reduction of the herniated bowel was carried out in the labor ward by the attending neonatologist; however, the herniated bowel reappeared when the infant started crying. At the end of the first week, surgical repair was successfully completed.

Comment

As the testes descend retroperitoneally from the genital ridge during the latter stages of embryonic life, the processus vaginalis, an outpouching of peritoneum, precedes the testes into the scrotum. The processus vaginalis originates at the internal ring, and as it leaves the canal at the external ring it turns inferiorly into the scrotum. The lower portion of this sac envelopes the testis to form the tunica vaginalis. The remainder of the sac normally atrophies before birth. The indirect

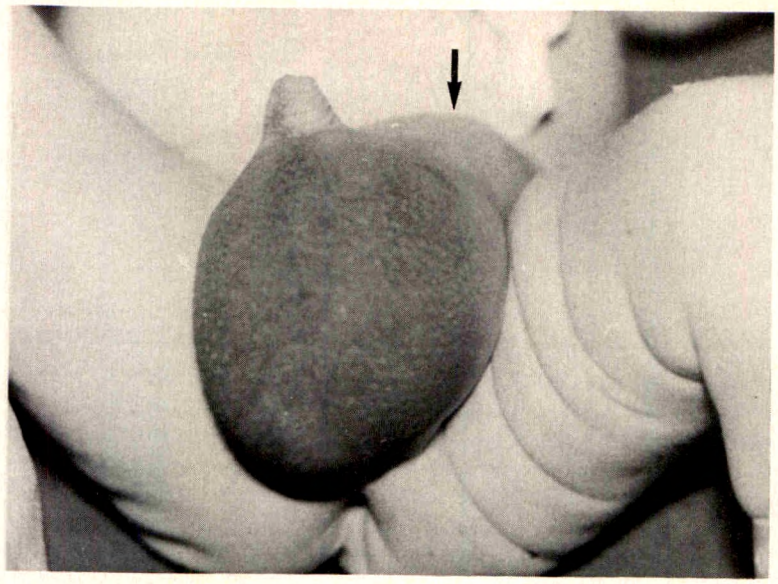


Fig. 3. Infant immediately after delivery. Huge scrotum is evident. Note bulging inguinal mass containing herniated small bowel *(arrow)*.

inguinal hernia results from a persistence of the processus vaginalis and manifests itself as a mass in the inguinal region when an abdominal viscus or peritoneal fluid is forced into it.

Although the hernial sac is present at birth, it usually remains empty for a variable period of time. By 2 or 3 months of age, when the infant is capable of increasing intraabdominal pressure, the sac may open and either fluid or abdominal content is forced into it. The hernial sac then appears as a swelling in the inguinal region, but it may extend in the male for a variable distance downward into the scrotum. In complicated cases a loop of bowel may become incarcerated in the sac.

The ultrasonographic markers for correct prenatal ultrasonographic diagnosis of inguinal scrotal hernia include the following: (1) huge scrotum, (2) cystic, fluid-containing structures inside the scrotum representing bowel loops, (3) evidence of peristalsis in the bowel, and (4) close relationship of both testicles. Because scrotal hernias in fetuses are rare, intraabdominal pathologic cause of elevation in abdominal pressure should be excluded first. Fetuses with multiple congenital anomalies, particularly those involving the lower abdomen, may display inguinal hernias as part of the anomaly complex.

An inguinal hernia extending into the scrotum should be carefully differentiated from a congenital hydrocele.[2] This differentiation is simpler in fetuses than in infants. In hydrocele the testicles are easily recognized because they are in clear peritoneal fluid, which gives a characteristically echo-free picture. This is not the case with scrotal hernia, in which the scrotum is filled with a partially echogenic mass with evident peristalsis.

Addendum

After submission of this article for publication, Ober et al.[3] reported a similar case.

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Uterine and umbilical flow velocity waveforms in normotensive and hypertensive subjects during the angiotensin II sensitivity test

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The systemic and uterine circulatory effects of angiotensin II infusion in five normotensive and 10 hypertensive subjects at the end of the third trimester of gestation were studied. Color Doppler ultrasonography was used for investigating the systolic/diastolic ratios on flow velocity waveforms in the uterine and umbilical arteries. A significant increase was observed in the systolic/diastolic ratio in the uterine artery in both groups. In normotensive subjects a differential effect of angiotensin II on the systemic diastolic blood pressure and on the systolic/diastolic ratio was observed; the uterine circulation had a slower response and a faster recovery than the maternal diastolic blood pressure. In hypertensive subjects this relative refractoriness to angiotensin II was less marked; the increase in uterine vascular resistance was faster and the recovery slower than in the normotensive group. No changes in the systolic/diastolic ratio in the umbilical artery were observed in either group. These results indicate that the uterine circulation of normotensive subjects is remarkably more refractory to angiotensin II than the circulation of hypertensive subjects. (AM J OBSTET GYNECOL 1992;166:910-6.)

Key words: Angiotensin II sensitivity test, Doppler ultrasonography, flow velocity waveform, pregnancy-induced hypertension, umbilical artery, uterine artery

An increased peripheral sensitivity of blood vessels to vasopressor substances is characteristic of pregnancy-induced hypertension. During pregnancy the refractoriness to angiotensin II is lost several weeks before clinical hypertension can be detected.[1] On the basis of these findings, an angiotensin II sensitivity test has been designed to predict the development of pregnancy-induced hypertension.[1] Although the physiologic basis of the angiotensin II test has been well investigated,[2] emerging ultrasonographic techniques offer the opportunity for a more profound investigation of the effects of the test. In our earlier study we observed a differential effect of angiotensin II on systemic and uterine circulation during the angiotensin II test in normotensive subjects at 24 to 26 weeks' gestation; the uterine circulation had a slower response and a faster recovery than the systemic diastolic blood pressure.[3] This study was designed to compare the effects of the angiotensin II test on maternal and fetal circulation in normotensive and hypertensive subjects during the third trimester of pregnancy. As in the earlier study, we applied color Doppler ultrasonography for vessel localization and pulsatile Doppler ultrasonography for recording uterine and umbilical flow velocity waveforms.

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Subjects and methods

Five normotensive healthy women with singleton pregnancies volunteered as a control group (group 1). The maternal age ranged from 22 to 31 years (mean 26.6 years) and weight from 69 to 87 kg (mean 79.0 kg); gestational ages ranged from 35 to 39 weeks (mean 37.2 weeks). The duration of pregnancy was ascertained by accurate menstrual history and a second-trimester ultrasonographic examination. Two patients were nulliparous and three patients were parous with uneventful previous pregnancies.

Group 2 consisted of 10 women with singleton pregnancies of 33 to 40 weeks' gestation (mean 37.0 weeks). The gestational age was estimated as described. The maternal age ranged from 17 to 34 years (mean 25.1 years) and weight from 64 to 87 kg (mean 78.9 kg). All subjects were nulliparous with no knowledge of a hypertensive disorder before pregnancy. Also all subjects were normotensive during the first and second trimesters of the pregnancy. The average duration of pregnancy at the time of the elevated blood pressure was 35.5 ± 2.3 weeks (mean ± SD) (range 31 to 39 weeks). All subjects were hospitalized because of a diastolic blood pressure >100 mm Hg on three or more measurements. After 2 to 4 days of hospital rest, the diastolic blood pressure still exceeded 100 mm Hg in all

cases. Six of the subjects had albuminuria (range 0.5 to 1.6 gm/L).

None of the subjects in either group used any medications except for iron supplementation. None were in labor or had uterine contractions during the investigation. All subjects gave written informed consent. The study protocol was approved by the Ethics Committee of the Turku University Hospital.

The angiotensin II sensitivity test was performed as previously described.[1, 3] The women were in the left lateral semirecumbent position in a quiet room. Maternal blood pressure and heart rate were automatically measured from the right arm at 1-minute intervals throughout the test by means of an oscillometer (sphygmomanometer, BP-203, Nippon Colin Co., Tokyo). An intravenous line for infusion was inserted in the left arm, and after the diastolic blood pressure had been stable for ≥20 minutes, the line was connected to an infusion pump with a syringe containing angiotensin II amide (1000 ng/ml in 0.9% sodium chloride solution, Hypertensin, Ciba-Geigy, Basel). The angiotensin II infusion was started at a rate of 1.5 ng/kg/min, and the dose rate was increased by 1.5 ng/kg/min at 4-minute intervals. Once a rise in diastolic blood pressure by 10 mm Hg and subsequently by 20 mm Hg was obtained and the rise was stable, the examinations for flow waveforms were performed. Thereafter the infusion was discontinued, and the maternal heart rate and blood pressure were recorded at 1-minute intervals during the 10-minute recovery period. The external fetal heart rate was continuously recorded with a Hewlett Packard model 8040 cardiotocograph (Hewlett-Packard, Böblingen, Germany).

Before the angiotensin II infusion was started, the flow velocity waveforms of the uterine and umbilical arteries were recorded by a color Doppler system with a 3.5 MHz transducer (model SSD 870, Aloka Co., Tokyo). A 100 Hz high-pass filter was used to remove signals from slow-moving tissues. In all cases the main branch of the uterine artery on the right side and the free-floating part of the umbilical artery were visualized easily with color, and the beam was directed to the vessel. Once 10 to 15 regular waveforms were recorded, the image was frozen and three waveforms from the uterine artery and four to five from the umbilical artery were manually analyzed; the maximum velocities during systole and diastole were obtained. Examinations of the umbilical artery were done only in the absence of fetal breathing or gross body movements. The peak-systolic and least-diastolic frequencies were measured and the systolic/diastolic ratio was calculated. The uterine artery flow velocity waveforms were obtained at the 10 and 20 mm Hg increments in maternal diastolic blood pressure and also at 1-minute intervals after the test until the systolic/diastolic ratio had returned to the initial level. The second Doppler examination of umbilical artery was made at the 20 mm Hg increment in maternal diastolic blood pressure. The correlation coefficient between repeated measurements of the uterine artery has been 0.91 (coefficient of variation ±2.5%) and for umbilical artery 0.97 (coefficient of variation ±2.0%).

Individual data were summarized as means and standard deviations. The blood pressure and systolic/diastolic ratio in the uterine artery were compared between the groups before the angiotensin II test by the Student *t* test for unpaired observations. Similarly, the heart rates and angiotensin II doses required for the 20 mm Hg pressure increase were compared in different groups by applying the Student *t* test for unpaired samples. The significance in the changes in the diastolic blood pressure, in the systolic/diastolic ratio of the uterine artery flow waveform velocity, and in the changes in the ratio of these parameters was tested by analysis of variance for repeated measurements with one between factor (group) and one within factor (time). When pooled orthogonal components showed nonsphericity, Greenhouse-Geisser adjusted probability values were used.[4] When a statistically significant group × time interaction or time effect was found, the analysis was continued by applying contrasts.

Results

In the beginning of the test, the diastolic blood pressure was 71 ± 8 mm Hg in group 1 and 97 ± 3 mm Hg in group 2 ($p < 0.001$). The baseline heart rates were 77 ± 11 and 82 ± 4 beats/min (not significant). At the peak of the diastolic blood pressure, they were 72 ± 10 and 75 ± 7 beats/min, respectively. The effective mean pressor dose of angiotensin II was significantly higher (11.9 ± 3.8 $ng/kg^{-1} \times min^{-1}$) in group 1 than in group 2 (7.1 ± 3.2 $ng/kg^{-1} \times min^{-1}$) ($p = 0.023$).

The systolic/diastolic ratio in the uterine artery at the beginning of the test was 1.59 ± 0.04 in group 1 and 1.84 ± 0.19 in group 2 ($p = 0.019$). After the rise in the diastolic blood pressure of 10 mm Hg, the systolic/diastolic ratios were similar (1.61 ± 0.05 and 1.85 ± 0.18). Once the increase by 20 mm Hg was reached, the systolic/diastolic ratio in both groups increased significantly ($p < 0.001$) to 1.91 ± 0.07 and to 2.27 ± 0.18, respectively. The mean fractional increases in systolic/diastolic ratio in the groups were not statistically different (20% ± 8% and 23% ± 7%, respectively).

The diastolic blood pressure decreased in both groups to the preinfusion level within 4 minutes (range 2 to 5 minutes) after the test. Fig. 1 shows the mean profile of the effect of angiotensin II infusion on the diastolic blood pressure and on the systolic/diastolic

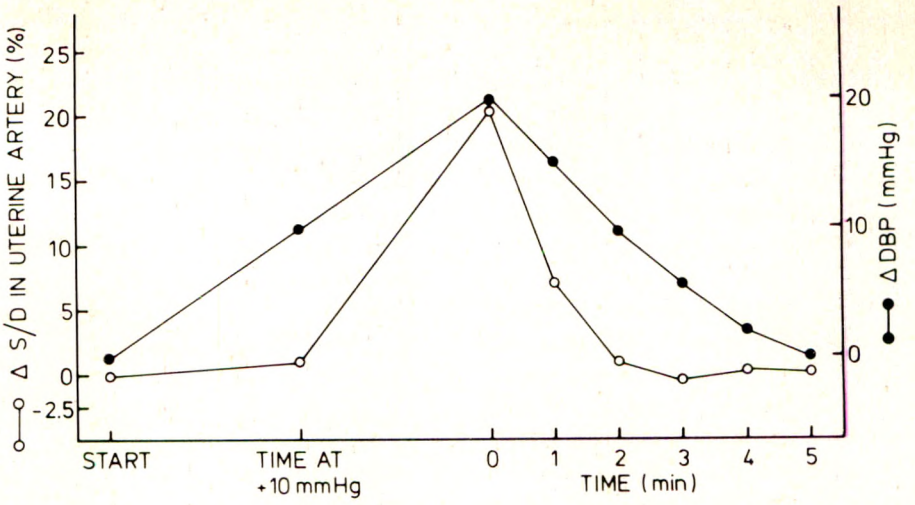


Fig. 1. Effect of angiotensin II sensitivity test on diastolic blood pressure (*DBP*, ●- - -● and systolic/diastolic ratio (*S/D*, ○- - -○) in flow velocity waveforms in uterine artery in normotensive subjects ($n = 5$).

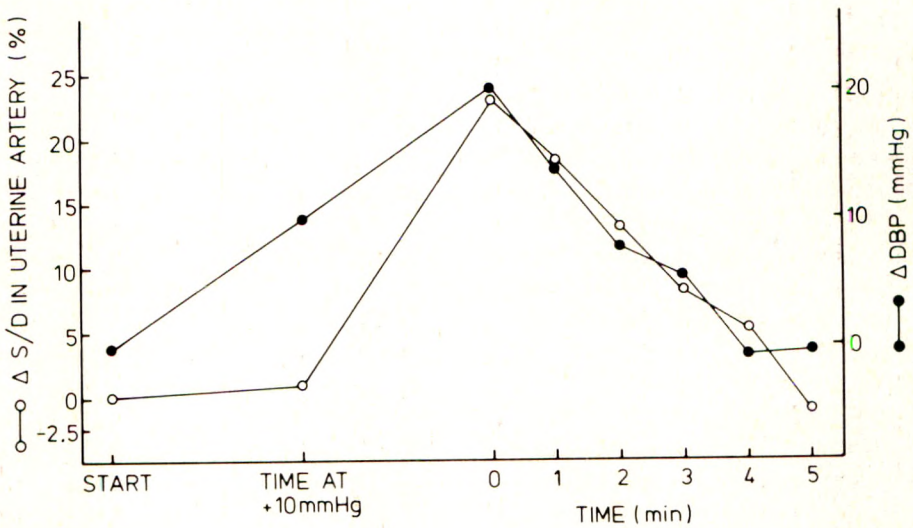


Fig. 2. Effects of angiotensin II sensitivity test on diastolic blood pressure (*DBP*, ●- - -●) and systolic/diastolic ratio (*S/D*, ○- - -○) in flow velocity waveforms in uterine artery in hypertensive subjects ($n = 10$).

ratio in uterine artery flow waveform velocity in group 1. Fig. 2 draws the mean profile of diastolic blood pressure and uterine artery flow velocity waveform in the group of hypertensive subjects (group 2). In normotensive women the response of the diastolic blood pressure and the systolic/diastolic ratio was significantly ($p < 0.001$) discordant (Table I). In contrast, in hypertensive women the declines of the diastolic blood pressure and systolic/diastolic ratio of the flow velocity waveform in the uterine artery were concordant (Table I). The comparison between the groups shows that the response of diastolic blood pressure to angiotensin II disappears at an equal rate in both groups (Table I). The recoveries of the increases in systolic/diastolic ratio in both groups were significantly faster in the normotensive group than in the hypertensive group (Table I). In the normotensive group the systolic/diastolic ratio in the uterine artery decreased faster than diastolic blood pressure and reached the initial level within 2 minutes in all subjects (Fig. 1). In group 2 the systolic/diastolic ratio in the uterine artery decreased to the preinfusion level within 4 minutes (range 3 to 5 minutes) (Fig. 2).

When the ratio of diastolic blood pressure to systolic/diastolic ratio is compared in the different groups, significant differences can be observed (Fig. 3, Table

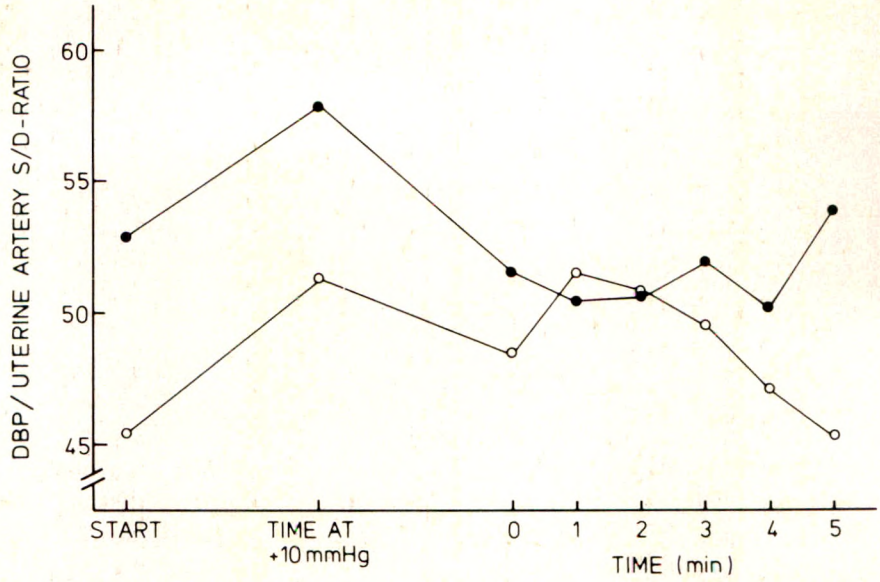


Fig. 3. Effects of angiotensin II sensitivity test on ratio of diastolic blood pressure *(DBP)* and systolic/diastolic ratio *(S/D)* in normotensive (○- - -○) and hypertensive (●- - -●) groups.

Table I. Comparison of time trend changes in diastolic blood pressure, systolic/diastolic ratio, and their ratio in normotensive (group 1) and hypertensive (group 2) subjects

	Diastolic blood pressure		*Systolic/diastolic ratio*		*Ratio of diastolic blood pressure and systolic/diastolic ratio*	
Group	*F test*	*p Value*	*F test*	*p Value*	*F test*	*p Value*
Group 1						
+10 mm Hg	768	<0.0001	0.36	0.56	29.41	<0.0001
+20 mm Hg	2166	<0.0001	79.05	<0.0001	9.46	0.0089
+1 min	73.47	<0.0001	6.91	0.0208	12.36	0.0038
+2 min	40.81	<0.0001	0.17	0.6857	20.21	<0.0001
+3 min	9.30	0.0093	0.09	0.7742	7.94	0.0145
+4 min	1.54	0.2370	0.00	0.9449	2.56	0.1336
+5 min	0.00	1.0000	0.01	0.9326	0.01	0.9246
Group 2						
+10 mm Hg	2009	<0.0001	0.18	0.6787	45.79	<0.0001
+20 mm Hg	4333	<0.0001	278	<0.0001	3.77	0.0742
+1 min	104	<0.0001	119	<0.0001	3.71	0.0764
+2 min	45.91	<0.0001	70.81	<0.0001	7.07	0.0197
+3 min	16.65	0.0013	21.71	0.0004	0.74	0.4050
+4 min	0.19	0.6684	5.02	0.0431	15.66	0.0016
+5 min	0.10	0.7518	4.56	0.0524	2.37	0.1480

Comparison was performed between every point of measurement against the initial value.

I). With an increase of diastolic blood pressure by 10 mm Hg, the ratio increased significantly ($p < 0.001$) and in a similar fashion in both groups. With an increase in diastolic blood pressure of 20 mm Hg, the ratio was still elevated in the normotensive group ($p = 0.009$); in the hypertensive group it returned toward the initial level ($p = 0.07$). Two ($p = 0.020$) and four ($p = 0.0016$) minutes after discontinuation of the infusion, the ratio between the diastolic blood pressure and the systolic/diastolic ratio was lower than the initial level in the hypertensive group, whereas in the normotensive group it was higher than initially until 4 minutes after cessation of the infusion.

When the groups were compared as a whole, the changes in diastolic blood pressure over time did not differ from each other ($p = 0.576$). Instead, both systolic/diastolic ratio ($p < 0.001$) and consequently the ratio of diastolic blood pressure to systolic/diastolic ratio ($p < 0.001$) differed significantly between the two groups (Table II).

Table II. Results of repeated measurements analysis of variance for diastolic blood pressure, systolic/diastolic ratio, and their ratio: Comparison of the groups

Variable	*Factor = Group*	*Factor = Time*	*Factor = Interaction*
Diastolic blood pressure			
F test	63.8	81.7	0.6
p Value	<0.001	<0.001	0.576
Systolic/diastolic			
F test	13.8	59.4	8.1
p Value	0.003	<0.001	<0.001
Ratio of diastolic blood pressure and systolic/diastolic ratio			
F test	1.3	10.0	8.9
p Value	0.273	<0.001	<0.001

The flow velocity waveform in the umbilical artery did not show any changes in either group. The mean systolic/diastolic ratios were 2.22 ± 0.09 (group 1) and 2.39 ± 0.21 (group 2) before angiotensin II infusion and 2.19 ± 0.16 and 2.39 ± 0.25, respectively, at the end of the test. The cardiotocograms showed, and the mothers felt, no uterine contractions during the tests. The fetal heart rate was normal and unaffected by the changes in the maternal diastolic blood pressure or by the changes in systolic/diastolic ratio in the uterine artery. Fetal movements were present in all cases at some time during the examination, and the fetal heart rate showed a reactive pattern.

Comment

The effects of angiotensin II on the systemic and uterine circulation during pregnancy have been investigated mainly in experimental animal models. By applying the Doppler method, it is possible noninvasively to study flow velocity waveforms whereby the end-diastolic frequencies of waveforms are likely to reflect the resistance of the downstream vascular bed. Very few direct comparisons between the actual flow and the simultaneous flow waveform velocity have been performed so far. In sheep fetuses the cotyledon resistance inversely correlates ($r = -0.94$) with diastolic/systolic ratio,[5] whereas systolic/diastolic ratio correlates well with umbilical vascular resistance ($r = 0.70$) and inversely with the umbilical artery flow ($r = -0.71$).[6] Also, in a computer model an increase in the uterine vascular bed resistance leads to a linear increase in the systolic/diastolic ratio.[7]

In animal studies a dual effect of angiotensin II on uterine blood flow has been observed. Although angiotensin II produces vasoconstriction in the uterine vasculature of pregnant sheep, the increase of the arterial perfusion pressure exceeds the effect of vasoconstriction, and consequently the uterine blood flow increases in the initial stage of infusion. When the uterine vascular resistance exceeds the effect of perfusion pressure, the uterine blood flow finally starts to decrease.[8, 9]

Our previous study group consisted of normotensive subjects, at 24 to 26 weeks' gestation,[3] which can now be compared with a group of normotensive subjects at 35 to 39 weeks. In the current group of normotensive subjects the diastolic blood pressure was slightly higher, the systolic/diastolic ratio slightly lower, and the dose of angiotensin II required in the test clearly lower than in the twenty-fifth week of pregnancy.[3] Very similar profiles in the diastolic blood pressure and systolic/diastolic ratio both in the initial phase and in the recovery phases were observed in both groups. In spite of the rise in the diastolic blood pressure by 10 mm Hg, the systolic/diastolic ratio barely changes and the ratio of diastolic blood pressure over the systolic/diastolic ratio increases significantly in both normotensive groups. This indicates a differential reaction in the systemic circulation and in the uterine circulation to angiotensin II. It also suggests that there is an increase in uterine blood flow in the earliest phase of infusion. Our results indicate that the human circulatory response to angiotensin II is similar to what has been observed in sheep models.

Another human study dealing with the flow velocity waveform measurements during the angiotensin test in human pregnancy is by Loquet et al.,[10] who studied normotensive women at 11 and 16 weeks of pregnancy. By using continuous Doppler, and in accordance with our study, they found an increasing pulsatility index in arcuate arteries with increasing diastolic blood pressure. Also in their study, in the early second trimester, the rise of the pulsatility index appears later than the blood pressure response.

In subjects with pregnancy-induced hypertension the pressor response to angiotensin II was stronger than in normotensive subjects. This is in accordance with earlier observations.[1, 11] Furthermore, the doses required for a rise in diastolic pressure of 20 mm Hg are very close to the doses at 37 weeks of gestation as reported by Gant et al.[1] The initial systolic/diastolic ratio in the uterine artery in hypertensive subjects was significantly higher than in normotensive subjects, reflecting an elevated resistance in the uterine vasculature in the pregnancy-induced hypertension.[12]

Very few studies are available on the uterine circulatory reactions to angiotensin II in hypertensive pregnancies. Lieb et al.[13] studied pregnant sheep with one-kidney hypertension. That model may differ from pregnancy-induced hypertension in humans, as the pressor effect of angiotensin II was actually less in hypertensive than in normotensive animals. Nevertheless, in both hypertensive and normotensive animals a differential effect on the systemic and uterine circulation was observed; with an increasing mean arterial blood pressure the uterine blood flow initially increased. By a totally different method, Gant et al.[14] observed a 13% decrease in the clearance rate of maternal plasma dehydroisoandrosterone sulfate during continuous infusion of angiotensin II. They suggested that this decrease could have occurred at least partly because of arteriolar vasoconstriction in uterine vasculature.

Our results show that the initial responses to angiotensin II in hypertensive and normotensive subjects were very similar. A relative refractoriness of the uterine vasculature to angiotensin II was observed in both groups at a 10 mm Hg rise in diastolic blood pressure. A difference between groups was observed when the diastolic blood pressure was raised by 20 mm Hg. In the group of normotensive subjects the ratio of diastolic blood pressure to the systolic/diastolic ratio was still significantly higher than the initial value; in the hypertensive group it had returned almost to the initial value. Hence it appears that the refractoriness of the uterine circulation and increased uterine blood flow prevailed longer in normotensive than in hypertensive subjects.

The same is also apparent from the recovery period; until the fourth minute of the recovery phase the ratio of diastolic blood pressure over the systolic/diastolic ratio was raised in the normotensive group. In contrast to this, the ratio in hypertensive group was similar to the initial level or significantly lowered during the entire recovery phase.

The reason for this different uterine vascular reactivity in normotensive and hypertensive subjects is open to speculation. Many observations indicate that vasodilating prostaglandins, prostacyclin in particular, may play an important role in attenuating the effect of angiotensin II.[15] In pregnancy-induced hypertension the elevated thromboxane A_2/prostacyclin ratio may interfere with vasodilation of the uterine vasculature.[16]

It has been suggested that high doses of angiotensin II given to the mother may have a direct or indirect depressing effect on fetal well-being.[17] Our previous[3] and current results show, however, that the angiotensin II sensitivity test is safe when performed in normotensive women between 24 and 40 weeks of gestation. It can also be safely carried out on subjects with moderate pregnancy-induced hypertension during the third trimester of pregnancy. An attenuated recovery of the uterine vascular reaction after angiotensin II infusion was, however, observed in hypertensive subjects. Although neither the flow velocity waveform nor fetal heart rate tracings showed deterioration of fetal well-being, it may, in fact, be compromised in women with more severe hypertension and an initially high systolic/diastolic ratio in the uterine artery flow.

In conclusion, the profile of systemic vascular reactivity to angiotensin II is similar in normotensive and hypertensive subjects, although the required dose of angiotensin II is higher in normotensive groups. The higher dose of angiotensin II needed in normotensive subjects may largely be due to relative refractoriness to angiotensin II in uterine vasculature, whereas the lowered dose requirement in hypertensive subjects reflects the loss of the refractoriness in uterine vasculature.

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Patterns of failure of bulky-barrel carcinomas of the cervix

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OBJECTIVE(S): This retrospective study was conducted to analyze the hypothesis that radiation therapy followed by extrafascial hysterectomy would improve survival in patients with bulky-barrel cervical carcinomas.

STUDY DESIGN: Forty-three patients with bulky-barrel carcinomas of the cervix were treated over a 14-year period. The majority of these were treated with approximately 4000 cGY external beam, followed by brachytherapy, followed by extrafascial hysterectomy.

RESULTS: Forty-seven percent of all patients are dead of disease; 2.3% are alive with disease. Of the total patients, 35% had diseased paraaortic nodes, and 80% of these are dead of disease. Of the patients dead of disease, 80% had distant metastases. Delayed complications included: vesicovaginal fistulas (n = 3), surgery for bowel obstruction (n = 3), rectovaginal fistula (n = 1), and vaginal vault necrosis (n = 3).

CONCLUSION: These data do not support an improvement in survival of patients with bulky-barrel–shaped lesions treated with irradiation plus adjunctive hysterectomy. (Am J Obstet Gynecol 1992;166:916-20.)

Key words: Cervical cancer; bulky-barrel lesions; adjuvant hysterectomy, complications

Early stage cervical cancers (IB, IIA) have been treated, with equal success, by radical surgery or by radiation therapy, each with a 5-year survival of approximately 80% to 90%. In contrast, the large, bulky-barrel lesions of the cervix have been associated with a poorer prognosis and a higher percentage of central treatment failures. In 1969, Durrance et al.[1] were the first to advocate combination therapy, consisting of radiation followed by an extrafascial hysterectomy to decrease the rate of central recurrence and to remove inadequately treated disease. These authors reported a significant decrease in central recurrences in patients with endocervical disease that expanded the cervix. Nelson et al.,[2] emphasized that a simple extrafascial hysterectomy should be done with no attempt to remove a 1 to 2 cm vaginal cuff. Removing the cuff was felt to be particularly hazardous, because any additional dissection of the bladder might result in a fistula. They also emphasized that a 6-week interval was needed between completion of the brachytherapy and hysterectomy. During this time span, further treatment regression may result in an almost normal cervix. However, delay much beyond 6 weeks may allow hard fibrosis, characteristic of late irradiation changes and vascular compromise. O'Quinn et al.[3] later analyzed the M.D. Anderson experience and noted several delayed complications in 79 patients undergoing this combined therapy. Maruyama et al.[4] reported an 84% 5-year survival rate and a 78% 10-year survival rate in patients treated with combined therapy. They noted that pa-

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tients with disease in the excised uterus had failure both in the pelvis and distantly. However, patients with negative specimens had failure in distant metastatic sites.

In a historic controlled study, Gallion et al.[5] compared 32 patients with stage IB barrel-shaped lesions treated by irradiation alone with 43 who were treated with irradiation followed by extrafascial hysterectomy. Recurrent cancer was noted in 47% of patients treated by irradiation alone, as compared with 16% of those treated with combined therapy. These authors reported no rectal or urinary tract fistulas in the patients treated with extrafascial hysterectomy. The validity of improved survival rates has been in doubt, because of the propensity of these tumors to spread to extrapelvic sites. Weems et al.[6] noted no difference in absolute survival in patients treated by irradiation alone or irradiation followed by hysterectomy. However, they noted a 2.5-fold increase in the percent of severe complications in the combined treatment group. Because of prior reports suggesting improved outcome in patients with bulky-barrel lesions, we began treating these patients with combined therapy in 1975.

The purpose of this article is to review the outcome of 43 patients with stage IB or IIA bulky-barrel lesions of the cervix, to analyze the failure sites, and to evaluate early and late complications in this select group of patients.

Material and methods

The clinical records from all cases with a diagnosis of carcinoma of the cervix at the Naval Hospital, Portsmouth, Virginia, from July 1975 through July 1984, and at the Medical College of Georgia, Augusta, Georgia, from July 1984 through July 1989, were reviewed. In all cases managed during this period of time the patients were examined by and operated on under the direction of one of the authors (D.G.G.). Follow-up information was obtained from tumor records and, when necessary, from phone calls and correspondence. All patients in this study were described at an informal or formal gynecologic-radiation-pathology tumor conference for final evaluation, review of pathologic diagnosis, and disposition. All pathologic specimens were reviewed by one of two pathologists (L.B.O. and R.L.S.) at their respective institutions. Recuts of slides were done when deemed necessary.

All patients underwent preoperative staging with an intravenous pyelogram, cystoscopy, and chest radiographs. Sixteen patients had a barium enema performed earlier in the study, and 10 patients had proctoscopy. Additionally, 35 patients underwent computed tomographic scan of the abdomen and pelvis with contrast. All patients were independently examined by at least two faculty physicians (gynecologists or radiation oncologists). Not all patients were examined while they were under anesthesia. If there was a disagreement in the examination, the lower stage (according to 1971 International Federation of Gynecology and Obstetrics classification) was assigned. Nine patients underwent a staging celiotomy before radiation. All nine had diseased paraaortic nodes and did not undergo hysterectomy. All patients in this study group retrospectively met current Gynecologic Oncology Group protocol requirements for bulky-barrel lesions. Patients either had an exophytic lesion with a diameter of ≥4 cm or had involvement of the cervix with cancer, resulting in expansion of the cervix and endocervix to 4 cm at the level of the internal os.

Patients who underwent hysterectomy were treated by a combination of irradiation and extrafascial hysterectomy. They received external beam therapy with 4000 to 4500 cGy followed by a cesium or radium insertion for 72 hours or a maximum of 5000 mg hours. Approximately 6 weeks after completion of the brachytherapy, patients underwent an extrafascial hysterectomy with bilateral salpingo-oophorectomy. Paraaortic nodes and pelvic nodes were assessed by careful palpation, and 77% of patients underwent paraaortic sampling. Patients with metastatic disease in paraaortic nodes were treated with 4500 cGy of extended field irradiation.

Results

During the period of study, 404 patients were treated for primary invasive cervical cancer. Forty-three patients (10.6%) had stage IB or IIA bulky-barrel lesions of the cervix. Five of the 43 were thought to have tumor encroaching on the vagina and were assigned as stage IIA.

The average age of the 43 patients was 45.7 years, with a range of 31 to 71. Twenty-nine percent were <40 years old, and 71% were <50. Seventy-one percent were white, 22% were black, and 7% were Malaysian. Most patients (89.5%) presented with some type of bleeding. None sought gynecologic consultation because of an abnormal cervical cytologic smear.

Cervical cytologic smears were suspicious for malignancy in 36 patients. Two had false-negative smears, and three did not have a smear. Three patients had a previous normal smear within a year before diagnosis, and seven, from 1 to 2 years. However, 16 patients had not had a smear for ≥5 years. All patients had normal pretreatment intravenous pyelograms and cystoscopic results. Only three patients with computed tomographic scans had suspicious nodes. One had a 2 cm pelvic node that was positive for cancer, as were the paraaortic nodes. The other two, both with suspicious 2 cm paraaortic nodes, had paraaortic node dissections with negative findings.

Lesion size or distension of the cervix was noted to be 4 cm in 19%, 5 cm in 37%, 6 cm in 17%, and >6 cm in 27%. When two patients alive with disease or

Table I. Survival for all patients*

Status	Patients	
	No.	%
No evidence of disease	21	48.8
Dead of disease	20	46.6
Alive with disease	1	2.3
Dead of intercurrent disease	1	2.3
TOTAL	43	100

All living patients had follow-up for at least 2 years.

Table II. Survival and cell type

Cell type	No. of patients	Dead of disease	
		No.	%
Squamous	29	10	34.5
Adenocarcinoma	9	5	55.5
Adenosquamous	4	4	100
Neuroendocrine	1	1	100
TOTAL	43	20	46.6

dead of intercurrent disease are excluded, no significant difference in survival was noted in those with lesions 4 to 5 cm in size (55%) as compared with those with lesions ≥6 cm (50%). Only six of the patients had an exophytic lesion with no apparent endocervical distension. The survival in these patients was similar to that of patients who had distension of the endocervix. Five of the 43 had clinical stage IIA assigned, and only one of these is alive.

None of the patients were lost to follow-up. All of the living patients have been evaluated for at least 24 months. Of the 43 patients, 47% are dead of disease (Table I). One patient is alive with disease. She has lung metastasis and is alive 36 months after diagnosis after cisplatin chemotherapy, lobectomy, and further chemotherapy. The patient dead of intercurrent disease died 50 months after original diagnosis of primary bronchogenic carcinoma of the lung. Tumors were histologically classified as squamous carcinoma (67%), adenocarcinoma (21%), adenosquamous carcinoma (10%), and neuroendocrine carcinoma (2%). Significantly, more patients with barrel lesions were dead of disease if the tumor had an adenomatous component, and no patients with adenosquamous cell tumor survived (Table II). Of the 10 patients dead of disease who had squamous tumor, the differentiation of the squamous cells did not affect survival.

Nine patients underwent a staging procedure and did not have hysterectomy. All nine had diseased paraaortic nodes. One of these patients is alive without evidence of disease 6 years later. She was treated with extended-field irradiation plus four cycles of vincristine, bleomycin, and cisplatin. Another of these nine had metastases to the lungs noted 2 years after extended-field irradiation. She was treated with cisplatin followed by mitomycin C and died 40 months after original diagnosis. Six patients had diseased paraaortic nodes resected at the time of hysterectomy. All were treated with extended-field paraaortic irradiation. Two of these are alive, 2 and 11 years after diagnosis. All but one of the patients with diseased paraaortic nodes had palpable nodes. Of the 43 patients, 15 (34.8%) had diseased paraaortic nodes, and 12 of these 15 (80%) are dead of disease.

Table III. Residual disease in excised cervix and survival

Amount	No. of patients	Dead of disease	
		No.	%
None*	15	3	20.0
Superficial Invasion	12	4	33.3
Deep invasion	7	5	71.4
TOTAL	34	12	35.3

*One patient is alive with metastatic disease, and one is dead of intercurrent disease.

Thirty-four of the 43 patients underwent hysterectomy after irradiation. Residual tumor deeply invading the excised, irradiated cervix was a poor prognostic factor, and 71.4% of such patients are dead of disease (Table III). If tumor was noted in vascular-like spaces in biopsy or hysterectomy specimens, 67% of the patients died, as compared with 31% without vascular-like invasion. Cell washings of the cul-de-sac were done in all 34 patients. Two (5.4%) had positive washings, and both died of disease.

Among patients dying of disease, 80% had distant metastases at the time of death (Table IV). Among patients dying of pelvic recurrence or pelvic recurrence plus distant metastases, 4 (36.4%) had initial recurrence on the pelvic sidewall, with no evidence of central disease. Distant metastases were documented by autopsy, radiography, computed tomography, and in one case by an upper gastrointestinal series showing involvement of the duodenum and proximal jejunum. Common sites of metastases included abdominal cavity, with or without small bowel involvement ($n = 9$); liver ($n = 7$); lung ($n = 7$); bone ($n = 2$); and supraclavicular nodes ($n = 3$). One patient each had metastases to the inguinal nodes, the heart, and the spleen. Of the 16 patients with distant metastases, 75% had previously noted diseased paraaortic nodes. Fifty-three percent of patients who died of disease did so within 12 months

Table IV. Patterns of recurrence in patients dead of disease

Site	Patients	
	No.	%
Distant metastases—all	16	80
Distant metastases alone	9	45
Distant and local metastases	7	35
Local alone	4	20
TOTAL	20	100

of the original diagnosis, and 77% died within 24 months.

Operative complications occurred in 9 of 43 patients (21%) undergoing any abdominal surgery, including 4 with wound infections, 3 with ileus, and 2 with pneumonia or atelectasis. However, 12 of the 34 patients eventually undergoing hysterectomy (35.3%) had significant delayed complications. All with delayed complications have no evidence of disease. Three were later operated on for small bowel obstruction, and two of these had small bowel resections. Three were treated conservatively for >8 months for vaginal vault necrosis. Vesicovaginal fistulas developed in 2, one had a rectovaginal fistula requiring colostomy, and one had a vesicovaginal fistula plus a rectovaginal fistula. Significant hemorrhagic cystitis developed in one patient, and another had peripheral neuropathy.

Comment

The mean age in this series was 45.7 years, similar to that reported in other series.[2, 3, 5] Blacks constituted 22% of the patients studied, which is a disproportionately lower proportion than the percentage of blacks in the patient population at the Medical College of Georgia. Frequent screening with cervical smears may have led to earlier diagnosis in some patients. It is noteworthy that 42% had not had a cervical cytologic smear in 5 years.

The overall survival of 80% to 90% in non-barrel, stage I to IIA cervical cancers treated with radical surgery or radiation alone is well known.[7, 8] However, Durrance et al.[1] and Gallion et al.[5] noted a significant reduction in survival rates to 53% and 64%, respectively, in comparably staged patients with the bulky-barrel–shaped lesions treated with irradiation alone. The addition of extrafascial hysterectomy to preoperative radiation has improved the survival rates, and in some series the survival rates are comparable to those of early stage carcinoma of the cervix[1, 2, 5, 9, 10] (Table V). In our series only 59% of patients are free of disease after combined treatment, significantly lower than the rates in most other reported series.

The survival for patients with adenosquamous cell lesions was dismal. We have previously reported a relatively poorer survival in patients with early stage adenocarcinoma,[11] and we separately reported a poor prognosis in patients with early stage adenosquamous cell carcinoma, independent of lesion size.[12] Russel et al.[9] also reported relatively more recurrences in patients with adenomatous-type lesions treated with combined modalities. In the current series no difference in survival was noted when different-sized lesions >4 cm were compared. Histologic examination of the hysterectomy specimens revealed residual tumor in 14 of 43 cases (33%) in the Kentucky series.[5] In our series residual tumor was found in 19 of 34 cases (56%). Twelve patients had superficial invasion, and seven patients had deep invasion. As expected, deeper invasion correlated with poorer prognosis. Russel et al.[9] also noted more recurrences if patients had residual tumor after preoperative irradiation.

Table V. Survival in patients treated by combined therapy

Author	No. of patients	Patients with no evidence of disease	
		No.	%
Durrance et al. (1969)	39	29	74
Nelson et al.[2] (1975)	50	37	74
Gallion et al.[5] (1985)	43	36	84
Russel et al.[9] (1987)	25	14	56
Tinga et al.[10] (1988)	20	16	80
Current series	34	20	59
TOTAL	211	152	72

The incidence of paraaortic lymph node metastases varies directly with tumor volume and has been reported to be as high as 20% in patients with stage IB bulky endophytic tumors.[13, 14] In our series the percentage of diseased paraaortic nodes was much higher, and these patients did poorly. Before any preoperative irradiation plan is instituted, strong consideration for surgical staging should be given in these high-risk patients.

In our series 45% of patients with recurrence had distant metastases alone, similar to the rate of Nelson et al.[2] (39%) and Gallion et al.[5] (38%). Additionally, we noted that 35% of patients had distant metastases plus pelvic recurrence. Gallion et al.[5] reported a pelvis-only recurrence rate of 13%, as compared with 46% for Nelson et al.[2] and 20% in our series. Note that among our patients with pelvic recurrence, four initially had recurrence on the sidewall. The high percentage of

patients described herein with recurrent disease outside the irradiated field is disturbing and should initiate trials of systemic therapy. Kim et al.[15] reported data evaluating the effect of preoperative chemotherapy on survival in patients with stage IB and IIB bulky tumors. The 54 patients first received initial combination chemotherapy of vinblastine, bleomycin, and cisplatin and later underwent radical hysterectomy with bilateral pelvic lymphadenectomy. The overall 2-year tumor-free survival rate was 94%. Of note, patients with lymph node involvement were treated with an additional three courses of chemotherapy, and patients with residual parametrial disease were treated with radiation therapy. In spite of the flaws of the study, their results are encouraging. Rettenmaier et al.[16] suggest an up-front surgical approach of radical hysterectomy with pelvic lymph node dissection, paraaortic node sampling, and tailoring postoperative radiation therapy to the extent of disease. Only 35% of the patients needed postoperative irradiation, and the projected 5-year survival rate for all patients was 79%.

Major complications for the combined approach have been reported by others. Nelson et al.[2] reported a major complication rate of 7.4% (7/95), including six fistulas and one small bowel obstruction. O'Quinn et al.[3] reported a major complication rate of 11%, including seven fistulas, one severe vault slough, and one small bowel obstruction. In our series the major complication rate is much higher (38%) and included three small bowel obstructions, three vaginal vault necroses, and four fistulas. To decrease the complications of rectovaginal or vesicovaginal fistulas, the insertion of an omental pedicle between the bladder and rectum, sutured to the vaginal apex, might improve the integrity of these local tissues.[17] Because of our noted complications of vesicovaginal fistulas, we drain the bladder 6 days after operation and prefer to use a suprapubic catheter.

We have noted a significant decrease in survival in patients with bulky-barrel lesions treated by combined therapy as compared with most other large series. The difference is probably best explained by the preponderance of patients dying of disease with tumor outside the irradiated field. Surgical staging should be strongly considered in patients with these bulky lesions. Because the vast majority of those dying of disease eventually have distant metastases, systemic treatment should be considered. Pilot studies are indicated, and randomized prospective studies should follow. Not only did we not note an improvement in survival in patients treated with combined therapy, we also found a relatively high rate of major complications. The benefits of combined therapy may not outweigh the risks.

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Normal fetal Doppler inferior vena cava, transtricuspid, and umbilical artery flow velocity waveforms between 11 and 16 weeks' gestation

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OBJECTIVES: Our objectives were to determine flow velocity waveform patterns in the fetal inferior vena cava and to relate these waveforms to transtricuspid and umbilical artery waveforms and fetal heart rate in early gestation.

STUDY DESIGN: Doppler waveforms were recorded in 40 normal fetuses at 11 to 16 weeks of gestation. Only transvaginal scanning was carried out at 11 to 12 weeks and only transabdominal scanning was used at 15 to 16 weeks.

RESULTS: The ratio of time velocity integrals of flow from the inferior vena cava during systole and early diastole, the percentage of reverse flow in this vessel, the pulsatility index from the umbilical artery, and the fetal heart rate were negatively correlated with gestational age. Peak E-wave and A-wave velocities and E/A ratios from the transtricuspid waveforms were positively correlated with gestational age.

CONCLUSION: Early normal pregnancies are associated with remarkable changes in fetal flow velocity waveforms at both the cardiac and the extracardiac level. (Am J Obstet Gynecol 1992;166:921-4.)

Key words: Doppler ultrasonography, fetal blood flow

Flow velocities in the inferior vena cava have been studied both in the adult[1] and in the fetus.[2] During the second half of normal pregnancy transabdominal Doppler ultrasonography has shown that waveforms from the fetal inferior vena cava are characterized by a two-component forward-flow pattern reflecting ventricular systole and early diastole (passive atrial filling) and a one-component reverse-flow pattern reflecting atrial contraction.[2] At the same time, flow velocity waveforms from the fetal internal carotid artery, descending aorta, and umbilical artery depict a low vascular resistance at the fetal cerebral trunk and umbilical-placental level.[3-5] In the growth-retarded fetus raised umbilical-placental vascular resistance[3-5] and increased reverse flow in the inferior vena cava[2] were established.

Transvaginal scanning greatly enhances the ultrasonographic image quality of the developing fetus. Recent studies with transvaginal Doppler ultrasonography at the cardiac[6] and extracardiac artery level[7] in normal late-first-trimester fetuses demonstrated flow velocity waveforms that are clearly different from those seen during the late second and third trimesters of pregnancy. In the umbilical artery end-diastolic velocities were nearly always absent. At the atrioventricular level peak E-wave velocities, representing early diastolic filling, were only 45% to 50% of peak A-wave velocities, representing atrial contraction. Because cardiac performance is also determined by preload, it was hypothesized that late-first-trimester fetuses would show inferior vena cava flow velocity waveforms that were different from those observed later in pregnancy.

The objective of the current study was to determine the flow velocity waveform patterns in the fetal inferior vena cava and to relate these waveforms to transtricuspid and umbilical artery waveforms and fetal heart rate (FHR) during late-first-trimester and early second-trimester pregnancies.

Material and methods

A total of 40 normal singleton pregnancies at 11 to 16 weeks of gestation (median 14 weeks) was studied. The protocol for the Doppler studies was approved by the hospital ethics review board. All pregnant women consented to participate in the study. Maternal age ranged between 18 and 37 years (median 28 years).

Ultrasonographic Doppler studies were performed with a Hitachi EUB 450 (Hitachi Medical Corporation, Tokyo). A combined transvaginal real-time and pulsed Doppler system with a carrier frequency of 3.5 MHz

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(real-time) and 6.5 MHz (Doppler) or a combined transabdominal real-time and pulsed Doppler system with a carrier frequency of 3.5 MHz (real-time) and 3.0 MHz (Doppler) was used, depending on fetal size and position relative to the ultrasonographic probe. Both systems operate at a power output of <100 mW/cm^2 spatial peak temporal average in imaging and Doppler modes.

The pregnancy duration was determined from the last menstrual period and confirmed by ultrasonographic measurements of the crown-rump length (11 to 12 weeks) or the biparietal diameter (13 to 16 weeks). Each woman was included in the study once. Doppler studies were performed by one examiner (J.W.W.). Flow velocity waveforms from the inferior vena cava were obtained in a sagittal view that included the fetal right atrium, right ventricle, and ascending aorta.[2] The sample volume was positioned over the inferior vena cava immediately proximal to the right atrium. Transtricuspid flow velocity waveforms were recorded from the two-dimensional image in the four-chamber view.[8] The sample volume was placed immediately distal to the tricuspid valve. At both recording levels the angle between the Doppler cursor and the assumed direction of flow was always ≤ 20 degrees and the sample volume length 0.1 to 0.3 cm. The flow velocity waveform in the umbilical artery was obtained from a free-floating loop of the umbilical cord.[9] For all recordings the high-pass filter was set at 100 Hz.

All Doppler studies were performed with the woman in a semirecumbent position and during fetal apnea. The total examination time was limited to 15 minutes in each instance. All flow velocity waveforms were recorded on hard copies. A microcomputer (Olivetti M24, Olivetti B.V., Leiden, The Netherlands) linked to a Graphics tablet was used for analysis of the Doppler recordings. Waveform analysis in the inferior vena cava consisted of calculation of (1) the ratio between the time-velocity integral during systole and the time-velocity integral during early diastole and (2) the time-velocity integral during reverse flow (atrial contraction), which is expressed as a percentage of total forward flow during systole and early diastole (passive atrial filling).[2] Peak velocities at E wave (passive atrial filling) and A wave (atrial contraction) and E/A ratios were calculated from transtricuspid flow velocity waveforms. Waveform analysis in the umbilical artery consisted of calculation of the pulsatility index,[10] which is derived by dividing the difference between peak systolic and end-diastolic velocity by the averaged velocity over the entire cardiac cycle. At all three recording levels, an average of three consecutive flow velocity waveforms with the highest velocity and similar appearance was used to establish each value.

Linear regression analysis was used to compare flow velocity waveform parameters with gestational age. Partial correlation coefficients,[11] after gestational age was controlled for, were computed to assess the relationships between the different flow velocity waveform parameters and between each of these parameters and FHR. Partial correlation coefficients were used to avoid two parameters becoming artificially correlated when both were correlated with gestational age.

Results

Transvaginal scanning was carried out in all women at 11 to 12 weeks, in 50% of women at 13 weeks, and in 20% of women at 14 weeks of gestation. Transabdominal scanning was performed in the remaining 50% of women at 13 weeks, in 80% of women at 14 weeks, and in all women at 15 to 16 weeks of gestation. Technically acceptable waveforms were obtained from the fetal inferior vena cava in all 40 women, at umbilical artery level in 35 women (87.5%), and at tricuspid valve level in 31 women (77.5%). Recording failures were not confined to a particular gestational age. Fig. 1 represents flow velocity waveforms from all three recording levels at 11 and 16 weeks of gestation, respectively. A statistically significant negative correlation with gestational age was established for the ratio of time-velocity integrals of flow from the inferior vena cava during systole to time-velocity integrals of flow during early diastole ($r = -0.74$, $p < 0.0001$), the percentage of reverse flow in the inferior vena cava ($r = -0.80$, $p < 0.0001$), the pulsatility index from the umbilical artery ($r = -0.74$, $p < 0.0001$), and the FHR ($r = -0.66$; $p < 0.0001$). FHR varied between 170 and 180 beats/min at 11 weeks and between 140 and 150 beats/min at 16 weeks of gestation. End-diastolic flow velocities in the umbilical artery were absent in all cases at 11 to 12 weeks and in 54% of cases at 13 to 14 weeks. They were present in all cases at 15 to 16 weeks of gestation. A statistically significant positive correlation with gestational age was found for peak E-wave velocities ($r = +0.83$, $p < 0.0001$), peak A-wave velocities ($r = +0.73$, $p < 0.0001$), and E/A ratios ($r = +0.73$, $p < 0.0001$) from transtricuspid waveforms. No correlation existed between the different waveform parameters or between each of these waveform parameters and heart rate, when gestational age was controlled for.

Comment

Combined two-dimensional real-time and pulsed Doppler ultrasonography has been used to establish flow data in human fetuses under physiologic and pathophysiologic circumstances. To our knowledge the current study is a first effort to document changes in flow velocity waveform characteristics at fetal inferior vena cava and tricuspid valve level in late-first-trimester

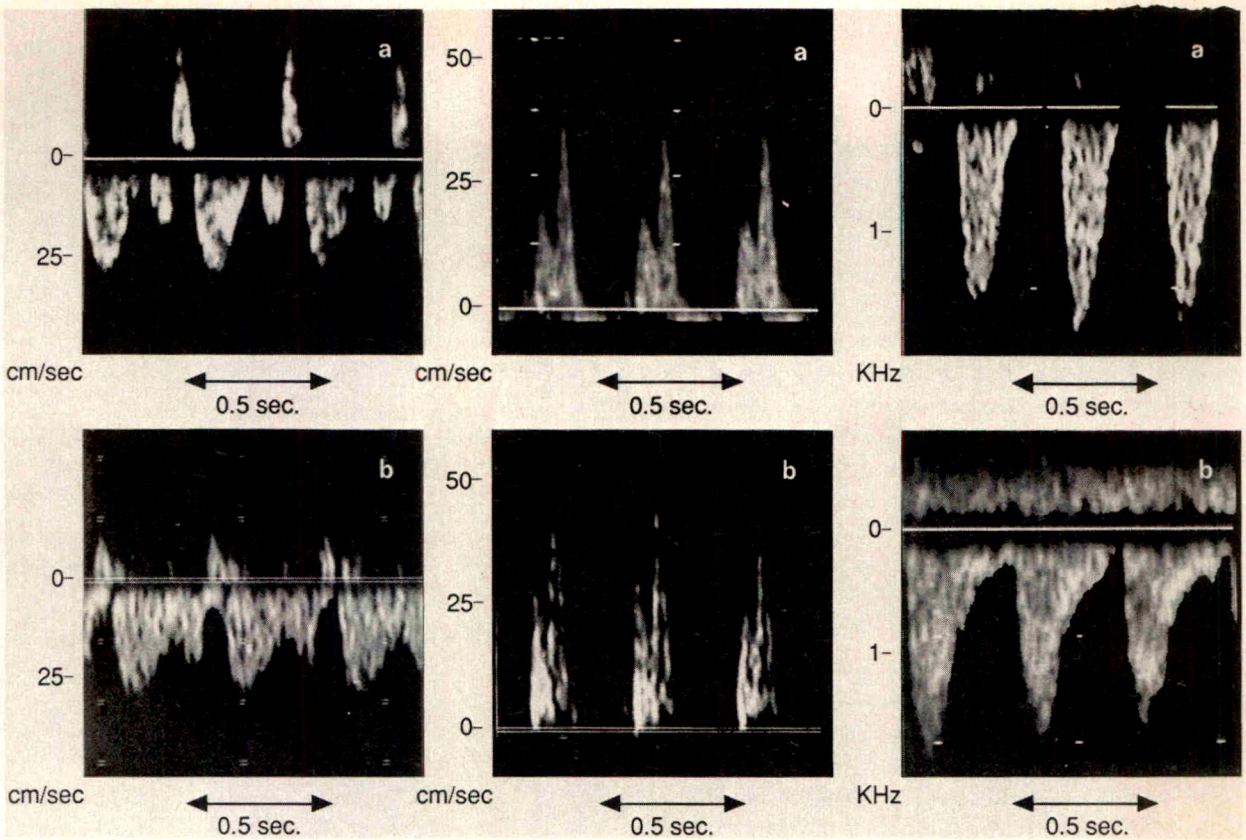


Fig. 1. Doppler flow velocity waveform tracings from inferior vena cava *(left panel)*, tricuspid valve *(center panel)*, and umbilical artery *(right panel)* at 11 weeks (*upper section*, **a**) and 16 weeks (*lower section*, **b**) of gestation.

and early second-trimester pregnancies. Transvaginal waveform recording became increasingly less successful after 12 weeks of gestation. This was mainly due to increasing fetal size resulting in large parts of the fetus becoming inaccessible to ultrasonographic imaging. For this reason transabdominal waveform recording was carried out in all cases after 14 weeks of gestation. Similar to those in late gestation, flow velocity waveforms from the fetal inferior vena cava in early pregnancy depict a two-component forward-flow pattern and a one-component reverse-flow pattern. Technically acceptable waveforms in this vessel were obtained in all instances, whereas this was not the case for waveform recordings at the other two scanning levels. This difference in recording rate was determined by the fact that the prime objective was to obtain acceptable waveforms from the fetal inferior vena cava. Because of the predetermined maximum study period of 15 minutes, this often resulted in less time being available for documentation of waveforms originating from the tricuspid valve level or umbilical artery.

Peak velocities at both E and A waves of transtricuspid waveforms display a marked rise with advancing gestational age, suggesting an increase in early diastolic atrial filling and active atrial contraction. This may be a result of raised cardiac compliance or increased ventricular relaxation rate. However, this cannot be substantiated because of lack of pressure data. At the same time there is a drop in ratios for time-velocity integrals of flow during systole to time-velocity integrals of flow during early diastole and a drop in percentage reverse flow in the fetal inferior vena cava. The lack of any relationship between inferior vena cava flow parameters and flow parameters originating from the other two measuring points, when standardized for gestational age, suggests a multifactorial cause of the observed venous waveform changes with advancing gestational age. Ventricular compliance, right-to-left atrial pressure differences, and cardiac afterload may play a role in this. Absolute values for percentage reverse flow in the fetal inferior vena cava at 11 to 12 weeks gestation are twice the values found at 16 weeks and four times the values established during late-third-trimester pregnancies.[12] Reed et al.[2] established that values for percentage reverse flow are the lowest in the heart rate range between 120 and 160 beats/min, which are nor-

mal rates for late-second-trimester and third-trimester pregnancies. During bradycardia (<120 beats/min) and tachycardia (>160 beats/min), a rise in percentage reverse flow in the inferior vena cava was noted, suggesting less optimal atrial contraction under these circumstances. In this study FHR was subject to a marked reduction, which has been attributed mainly to maturation of the parasympathetic system.[13] The absence of a correlation between heart rate and percentage reverse flow in the inferior vena cava suggests that heart rate is independent of the percentage reverse flow changes observed between 11 and 16 weeks of gestation. At the same time a marked reduction in pulsatility index in the umbilical artery was observed. This reduction was mainly determined by the appearance of end-diastolic flow, as from 13 to 14 weeks. This may be explained by a drop in umbilical-placental vascular resistance. In favor of this supposition is that this development coincides with a resurgence of endovascular trophoblast migration with a second wave of cells moving into intramyometrial layers of the spiral arteries. This will result in destruction of the medial musculoelastic tissue in the vessel wall and conversion of the thick-walled muscular spiral arteries into flaccid saclike uteroplacental vessels.[14, 15] In this way the placenta establishes its own low-pressure, high-conductance vascular systems, which are necessary to accommodate the increased blood flow to the developing fetus. It has also been demonstrated that this intravascular invasion by extravillous trophoblast is hampered in women who at a later stage have preeclampsia or intrauterine growth retardation.[16, 17] If there is a relationship between the aforementioned normal vascular changes at the placental level and the observed changes in fetal blood flow velocity waveforms in late-first-trimester pregnancies, then it would be of interest to examine fetal flow velocity waveforms in those early pregnancies that are at increased risk of subsequent development of preeclampsia or intrauterine growth retardation. Significant flow velocity waveform changes have been observed at umbilical-placental, cardiac, and cerebral levels under these pathologic circumstances in late gestation.[18]

It can be concluded from the current study that late-first-trimester and early second-trimester normal pregnancies are associated with remarkable changes in fetal flow velocity waveforms at both cardiac and extracardiac levels. These changes are independent of the reduction in FHR observed at this stage of early pregnancy. The reduction in inferior vena cava flow reversal may be a result of raised ventricular compliance or increased ventricular relaxation rate.

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Maternal glucose intolerance and the subcutaneous terbutaline pump

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OBJECTIVE: Our hypothesis was that use of the subcutaneous terbutaline pump does not affect maternal glucose tolerance.

STUDY DESIGN: With the 1-hour glucose tolerance test, we examined the incidence of glucose intolerance in 37 patients using the pump compared with that of 54 patients receiving oral terbutaline and 634 control subjects without risk factors for gestational diabetes. The frequency of gestational diabetes and the need for insulin to maintain glycemic control were subjected to χ^2 analysis.

RESULTS: The incidence of gestational diabetes was 6% in the control subjects, 5% in patients using the pump ($p = 0.8$), and 11% in those on the oral therapy regimen ($p = 0.4$). A total of 8% of controls who had gestational diabetes required both insulin and diet, compared with 100% using the pump ($p < 0.01$) and 50% on the oral terbutaline regimen ($p = 0.03$).

CONCLUSION: The incidence of gestational diabetes is not increased in patients receiving terbutaline via the subcutaneous pump. The use of terbutaline by any route significantly increases the need for insulin to achieve glycemic control. (Am J Obstet Gynecol 1992;166:925-8.)

Key words: Terbutaline, tocolytics, glucose intolerance, premature labor

Premature labor and delivery continue to contribute significantly to perinatal morbidity and mortality. The β-adrenergic receptor agonist class of tocolytic agents has long been used in the treatment of premature labor. Lack of pure β_2-adrenergic receptor selectivity results in many of the side effects often encountered in patients taking these medications. The long-term use of oral terbutaline in the treatment of preterm labor has been reported to result in maternal glucose intolerance.[1, 2] The subcutaneous terbutaline pump as described by Lam et al.[3] is a relatively new tocolytic modality that provides a continuous low-dose infusion of terbutaline, augmented with intermittent higher-dose boluses. The effect of this delivery system on glucose homeostasis has not been studied; therefore we examined the development of gestational diabetes in patients receiving subcutaneous terbutaline therapy.

Material and methods

All patients enrolled in this study received follow-up by the Division of Maternal-Fetal Medicine of the Hospital of the University of Pennsylvania. This study was approved by the Institutional Review Board of the University of Pennsylvania Medical Center. The study group included 91 consecutive private patients over a 24-month period between 26 and 36 weeks' gestation who were admitted to the labor floor with a diagnosis of premature labor. This diagnosis was based on documented cervical change or six or more uterine contractions per hour that were unresponsive to bed rest and intravenous hydration. The control group consisted of 634 consecutive patients whose pregnancies were uncomplicated by premature labor and with a gestational age at delivery ≥37 weeks. Patients with a history of pregestational or gestational diabetes, macrosomia, current steroid therapy, or multiple gestation were excluded from the study.

Study patients who required <24 hours of intravenous tocolysis with magnesium sulfate or ritodrine were subsequently placed on a regimen of oral therapy with 5 mg terbutaline every 4 hours. Patients who required >24 hours of intravenous tocolysis, multiple courses of intravenous tocolysis, or multiple-agent therapy were placed on the subcutaneous terbutaline pump (Model 404 SP, MiniMed Technologies, Sylmar, Calif.). The initial basal rate of delivery of terbutaline was calculated on the basis of pharmacokinetic parameters, including surface area, bioavailability, volume of distribution, clearance, elimination, and drug half-life.[4]

Both study and control patients were screened for glucose intolerance at 24 to 28 weeks' gestation with a standard 50 gm glucose load. All patients on a terbutaline regimen, both oral, and pump infusion, had evidence of a normal 1-hour oral glucose tolerance test

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Table I. Demographics

	Pump	*Oral*	*Control*
Maternal age at delivery (yr)	32.8 ± 2.8	32.1 ± 2.7	33.6 ± 4.8
Estimated gestational age at start of therapy (wk)	29.4 ± 1.5	28.9 ± 1.9	—
Estimated gestational age at end of therapy (wk)	35.6 ± 1.1	36.2 ± 0.9	—
Estimated gestational age at delivery (wk)*	36.6 ± 1.2	37.9 ± 1.3	38.2 ± 2.8
Birth weight (gm)†	3017.3 ± 303.3	3228.6 ± 583.5	3325.4 ± 487.2

*$p < 0.01$ (Student t test), pump versus control and pump versus oral.
†p = NS (Student t test).

Table II. Incidence of gestational diabetes

	Normal (1 hr GTT)		*Abnormal*			
			1 hr GTT		*3 hr GTT*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Control (n = 634)	520	82	114	18	36	6
Pump (n = 37)	30	81	7	19	2	5
Oral (n = 54)	40	74	14	26	6	11
p Value	NS		NS		NS	

NS, Not significant.

(GTT) before inclusion in the study. Patients receiving terbutaline were also tested with a 50 gm oral GTT 72 hours after initiation of therapy. Screening for gestational diabetes was continued in patients receiving oral therapy with a postprandial capillary glucose fingerstick every 1 to 2 weeks. Patients on the subcutaneous pump regimen were evaluated weekly in the same manner. Any 2-hour postprandial capillary glucose value >130 mg/dl was followed with a 50 gm oral GTT screen. All patients with an abnormal oral GTT result, defined as a serum glucose level >135 mg/dl, underwent a 3-hour GTT as described by O'Sullivan and Mahan.[5] The diagnosis of gestational diabetes was made when two or more abnormal values were found. All patients with gestational diabetes, in whom adequate glycemic control was not achieved with diet (fasting blood glucose level >100 mg/dl and/or 2-hour postprandial blood glucose level >120 mg/dl) were also treated with insulin. Statistical evaluation of the data obtained was performed with the Student t test, χ^2, and Fisher's exact test.

Results

Ninety-one consecutive patients whose pregnancies were complicated by premature labor were enrolled in the study. There was no difference in predisposing factors to gestational diabetes in any of the groups. In the control group the mean maternal age at delivery was 33.6 ± 4.8 years (mean ± SD), compared with 32.8 ± 2.8 years in those receiving pump therapy and 32.1 ± 2.7 years for those receiving oral therapy (Table I). The subcutaneous pump was instituted in 37 patients at a mean gestational age of 30.1 ± 2.1 weeks and discontinued at a mean of 35.9 ± 1.1 weeks. The mean gestational age at delivery was 37.2 weeks and average birth weight was 3228.6 ± 583.5 gm. The mean initial basal infusion rate was 0.056 ± 0.008 mg/hr. The mean final basal rate was 0.074 ± 0.02 mg/hr (Fig. 1). In addition, each patient had six to 10 scheduled or emergent daily boluses, each consisting of 0.25 mg terbutaline.

Fifty-four patients had successful management with oral terbutaline. The mean gestational ages at the start and cessation of oral therapy were 28.9 ± 1.96 and 36.2 ± 0.87 weeks, respectively. The mean gestational age at delivery was 37.9 ± 1.3 weeks with mean birth weight of 3017.3 ± 303.3 gm. The mean age at delivery for the control group was 38.2 ± 2.8 weeks with mean birth weight of 3325.4 ± 487.2 gm.

Seven patients (19%) receiving terbutaline pump therapy had an abnormal 1-hour GTT result compared with 114 patients (18%) in the control population (p = 0.9) and 14 patients (26%) receiving oral terbutaline (p = 0.6) (Table II). There was not a significant difference when those receiving oral therapy were compared with the control group (p = 0.2, χ^2).

Approximately 75% of the abnormal 1-hour GTT results in the terbutaline-treated patients occurred 72 hours after the medication was started. Abnormalities in an additional 25% were diagnosed within 2 weeks of initiation of therapy. In all patients with development of glucose intolerance, this occurred before 35 weeks' gestational age. An abnormal 3-hour GTT result was observed in two patients receiving subcutaneous terbutaline therapy (5%) compared with 36 patients (6%) in the control population (p = 0.8, χ^2) and six patients (11%) receiving oral medication (p = 0.4, χ^2). Although gestational diabetes developed in nearly twice the percentage of patients receiving oral therapy when compared with the controls, this difference was not significant (p = 0.1, χ^2).

The incidence of macrosomia in the controls with gestational diabetes was 5%. There were no cases of macrosomia in either group treated with terbutaline.

The treatment modalities needed to achieve adequate glycemic control in all patients are described in Table III. Both of the patients receiving subcutaneous terbutaline infusion who had gestational diabetes required insulin, compared with 8% in the control group

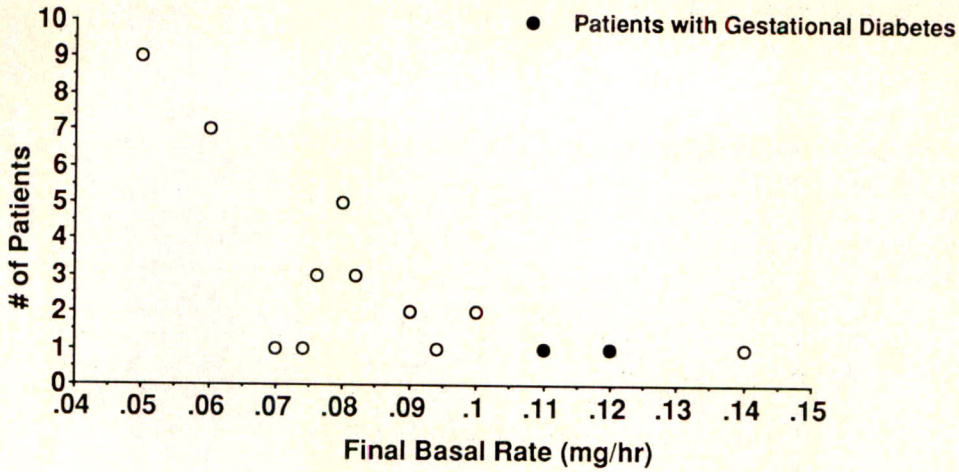


Fig. 1. Final terbutaline basal rate infusion.

($p < 0.01$, χ^2). A significant difference was also noted when those patients receiving oral therapy were compared with the control population ($p = 0.03$, χ^2).

Comment

The β-sympathomimetics are among the most common tocolytic agents used in the management of preterm labor. Long-term terbutaline use in pregnancy has been associated with a variety of maternal side effects, including electrolyte imbalance[6] and cardiovascular changes.[7] The development of tachyphylaxis with long-term terbutaline therapy is well documented.[8, 9] Several studies have indicated an association between terbutaline and the development of maternal glucose intolerance.[1, 2] The β-adrenergic receptor agonists have been shown to stimulate glucagon production by the pancreas, as well as increase gluconeogenesis and glycogenolysis.[10]

We did not find a statistically significant difference in the incidence of abnormal 1-hour screening with 50 gm of glucose between any of the groups either at initial evaluation or on start of therapy. There was no significant difference noted in the incidence of gestational diabetes when patients on the terbutaline pump regimen were compared with the control population or to those receiving oral therapy. Given the 80% sensitivity of the 1-hour 50 gm glucose screen,[11] it is possible that gestational diabetes would have developed in seven additional patients in the total population. It is unlikely that all would have come from the group with preterm labor who required terbutaline therapy.

Contrary to previous studies, we did not find a significant difference in glucose intolerance when patients receiving oral medication were compared with the control group ($p = 0.1$). The biggest difference between our study and the others is that all of the terbutaline-treated patients in our population had a previously normal 1-hour GTT result. Since the terbutaline-treated patients studied by Main et al.[1] and Angel et al.[2] were not all prescreened for glucose intolerance, the larger number of patients with an abnormal GTT result may include those patients who were destined to have glucose intolerance even without the added stress of a β-sympathomimetic. Our incidence of gestational diabetes in those patients receiving oral terbutaline (11%) agrees quite well with that found by Angel et al.[2] (11.6%). Main et al.[1] did describe nine patients who had normal glucose test results before treatment with terbutaline. We found that 6 of 54, or 1 of 9 patients receiving oral terbutaline, had gestational diabetes when compared with 2 of 9 patients in the prior study by Main et al.[1] It may be that the smaller numbers involved in these studies makes this difference appear greater than it really is.

The difference between oral and subcutaneous terbutaline may reflect the difference in dosing between the two tocolytic modalities. Lam et al.[3] have determined that a very low continuous basal infusion rate of 0.05 mg/hr will suppress uterine contractility. By using the subcutaneous pump, each patient receives a total daily dose of ≤3 mg/day compared with an oral dose

Table III. Methods of glycemic control in gestational diabetes

	Insulin vs diet				
	Diet		*Diet and insulin*		*Total*
	No.	*%*	*No.*	*%*	*No.*
Control	33	92	3	8	36
Oral	3	50	3	50*	6
Pump	0	0	2	100†	2

*$p < 0.05$, oral versus control (χ^2).

†$p < 0.01$, pump versus control; p = not significant, pump versus oral (Fisher's exact test).

of at least 30 mg/day. There are, of course, different absorptions with the two routes of administration, and therapeutic serum terbutaline levels have not yet been determined for the two groups. It has been postulated that the lower basal concentration of terbutaline supplemented by intermittent boluses can decrease the tachyphylaxis noted with the higher oral doses. In addition, the lower daily terbutaline dose given with the pump may result in less maternal gluconeogenesis and glycogenolysis, resulting in a lower incidence of carbohydrate intolerance. The two patients with development of gestational diabetes were maintained at a basal rate that was higher than average (Fig. 1).

An interesting finding was the significant difference in the need for insulin therapy to achieve adequate glycemic control in patients receiving terbutaline therapy when compared with controls. Main et al.[1] have previously described an increased need for insulin in patients receiving oral terbutaline therapy. Although our data indicate that this may also be true for patients using the terbutaline pump, many more patients will need to be observed for confirmation. The effect of the β-sympathomimetics may cause a more profound derangement of glucose homeostasis than that seen in idiopathic gestational diabetes.

In summary, the incidence of maternal glucose intolerance does not appear to be increased in those patients using the subcutaneous terbutaline pump even though testing is carried out more frequently after medication is started. However, the use of terbutaline, both orally and with the pump, appears to increase the need for insulin in those patients with development of gestational diabetes. Although the development of glucose intolerance in terbutaline-treated patients does not appear to be statistically different, this should not imply that there is not a clinical significance. The 5% to 10% incidence of gestational diabetes in those patients suggests that frequent surveillance of glucose tolerance, even in those previously tested, is recommended when they are receiving any form of terbutaline therapy.

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Hydrops fetalis associated with congenital myotonic dystrophy

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We report a case of congenital myotonic dystrophy in a newborn infant who presented with hydrops fatalis. Clinical features were hypotonia, generalized edema, pleural effusion, respiratory distress, scalp hematomas, and tented mouth facies. Review of literature shows that congenital myotonic dystrophy is not a rare cause of nonimmune hydrops fetalis as previously thought. (Am J Obstet Gynecol 1992;166: 929-30.)

Key words: Nonimmune hydrops fetalis, congenital myotonic dystrophy

Various maternal and fetal conditions are associated with nonimmune hydrops fetalis. Failure to identify a causative agent remains a frequent dilemma because up to 30% of cases are called "idiopathic."

Congenital myotonic dystrophy is an autosomal-dominant muscle disorder that is rarely included among conditions associated with nonimmune hydrops fetalis. This report describes a case of congenital myotonic dystrophy presenting as fetal hydrops.

Case report

A 2760 gm, female infant was born at 36 weeks' gestation to a 33-year-old multigravid mother with type A, Rh-positive blood. The mother had a history of slowly progressive lower-extremity weakness for the past few years. There was no family history of neuromuscular diseases. Previous pregnancies were uneventful and resulted in two healthy male children. The current pregnancy was complicated by worsening muscle weakness and progressive third-trimester polyhydramnios. The infant was delivered by cesarean section because of breech presentation. Post partum the mother had respiratory failure and required mechanical ventilation for a few days. The infant had Apgar scores of 2 and 5 at 1 and 5 minutes. Immediate resuscitation was required with intubation for poor respiratory effort. The infant remained severely hypotonic and continued to require mechanical ventilation. Generalized body edema was seen more extensively over the head and neck area. A "tented-shape" mouth was noted. Chest radiography revealed a right-sided pleural effusion and thin gracile ribs. Extensive scalp hematoma with no evidence of blood dyscrasia developed in the second week of life. Subsequently, edema and pleural effusion resolved and she was weaned from mechanical ventilatory support by 3 weeks of age. Her initial feeding difficulty slowly improved and she was feeding normally by the time of discharge home at 2 months of age.

The infant's blood type was A, Rh-positive and Coombs test negative. Karyotype was normal. A workup for congenital infection (TORCH [toxoplasmosis, rubella, cytomegalovirus, and herpes simplex] and parvovirus) was negative. Urine and serum screen for metabolic diseases gave normal results. The serum creatine phosphokinase level was within the normal range. Initial chest sonogram showed a right pleural effusion and a poorly moving and elevated right hemidiaphragm. The diagnosis of congenital muscular dystrophy was suspected by the characteristic maternal myopathic facies and myotonic grip. The mother's diagnosis was confirmed by neurologic consultation and abnormal results of electromyographic studies.

Comment

Congenital myotonic dystrophy is rarely listed among the conditions associated with nonimmune hydrops fetalis. One recent study mentioned one case among 900 patients with nonimmune hydrops fetalis.[1] However, in the pediatric literature we could identify at least 10 cases of congenital myotonic dystrophy with hydropic features.[2-4]

Pregnancy with an affected infant is often complicated by polyhydramnios, decreased fetal activity, breech presentation, and prematurity. A spectrum of neonatal presentations with congenital myotonic dystrophy may range from the fetus who is lost in early abortion, through the preterm baby who dies soon after birth, to the term asymptomatic infant. Early neonatal diagnosis of congenital myotonic dystrophy is difficult and many affected infants may die early in life before the diagnosis can be made. In addition, clinical presentation is variable and nonspecific, and severely affected babies can be misdiagnosed as having perinatal asphyxia.

Congenital myotonic dystrophy is a challenging di-

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agnosis that can be suggested by careful clinical evaluation of the mother and her newborn. In fully expressed disease the mother has a characteristic myopathic facies with hollow temples and myotonic grip. On the other hand, the mother may be so mildly affected that she is unaware of the disease. A negative family history does not rule out the possibility. Both muscle biopsy and electromyography give only suggestive evidence, and necropsy examination is nonspecific.

Recently, localization of the congenital myotonic dystrophy gene on chromosome 19 with tightly linked deoxyribonucleic acid probes has facilitated prenatal diagnosis.

Awareness of the association between congenital myotonic dystrophy and nonimmune hydrops fetalis is important to reduce further the proportion of idiopathic nonimmune hydrops fetalis because congenital myotonic dystrophy might be a more frequent etiologic factor than previously thought.

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Vaginal injury resulting from sliding down a water chute

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A 27-year-old woman was admitted to the hospital because of profuse vaginal bleeding from a vaginal laceration caused by the impact of water to the perineum after sliding down a water chute. At the time of the accident the patient was using a vaginal tampon. The potential mechanism for this trauma is discussed. (Am J Obstet Gynecol 1992;166:930-1.)

Key words: Vaginal trauma, water chute

A case of profuse vaginal bleeding caused by a slide down a water chute is described.

Case report

A 27-year-old healthy woman, gravida 1, para 1, was initially seen at the emergency department with massive vaginal bleeding after trauma to the lower back and vulva. This occurred when she hit the water after sliding down a water chute.

On admission the patient was conscious, pale, and had tachycardia to 120 beats/min.

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General examination revealed no special pathologic condition. The abdomen was soft without tenderness or guarding, but there was severe vaginal bleeding, tenderness, and swelling in the sacral area and the vulva. The patient advised that because she had been due to menstruate she had inserted a tampon that was present at the accident. However, she had removed the tampon before arriving at the hospital.

On arrival in the emergency room, an intravenous line was established with lactated Ringer's solution. Hemoglobin count was 12 gm/dl, and hematocrit was 34%. After the vagina was cleared of clots, the source of the bleeding was discovered to be a crescent-shaped laceration high in the left fornix. The patient was immediately transferred to the operating room and given a general anesthetic. A vaginal tear approximately 7 cm long that extended from the left lateral fornix to the posterior fornix was found. There were a few more superficial vaginal lacerations. The tear was sutured with single stitches of 1-0 catgut.

After the repair was completed, hemoglobin count was 9.1 gm/dl, with hematocrit of 27%.

The patient was discharged 2 days later in good condition.

Comment

In the literature there are few reports regarding vaginal tears resulting from waterskiing. Women who are active in water sports such as waterskiing are potentially susceptible to injuries that include sprains, limb fractures, sinusitis, trauma to the tympanic membrane, and injuries to the urethra and urinary bladder.[1] To the best of our knowledge there is no report about vaginal tears after sliding down a water chute.

A review of case reports about vaginal tears that occurred while the patient was waterskiing show that the accident occurs in the first stage of skiing: The skier is still in the water, her legs in front of her, and as she is pulled forward by the accelerating boat, water that is under high pressure hits the perineum.

The normal, unaroused vagina is a potential space that is H-shaped when viewed end-on with the anterior and posterior wall opposed.

Matsala[2] reviewed traumatic injuries to the vagina during sexual intercourse and reported that most of the tears are located in the upper two thirds of the vagina. During intercourse this part of the vagina stretches to 20% to 30% more than its usual length; its inner diameter increases twofold to threefold. The uterus is pulled from its usual location, causing a change in the anatomic relationships within the pelvis and in vaginal symmetry. With our knowledge of sexually related trauma, we can attempt to explain the mechanism by which our patient sustained her injuries.

When a woman slides down a water chute, her uterus is tilted forward because of the acceleration, thus stretching the vaginal wall. If her legs are abducted, the brunt of the water's impact will be to the perineum. Water, under high pressure, enters the vagina, dilates its walls, and causes injury to the upper two thirds of the vagina. Moreover, our patient wore a vaginal tampon, which may have had a pistonlike effect that added to the stretching forces on the vagina.

Treatment includes the control of bleeding, treating hemorrhagic shock if present, repairing the tear, and preventing possible infection by giving the patient antibiotics.

We recommend that women who use water chutes be instructed to slide down with their legs adducted, so that the feet, not the perineum, will be the first to hit the water. Moreover, women should not use tampons while engaged in this activity.

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Fetal urine biochemistry in the assessment of obstructive uropathy

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In 60 fetuses with obstructive uropathy, sodium, total calcium, urea, and creatinine were measured in samples obtained by "urodochocentesis" or pyelocentesis at 16 to 36 weeks' gestation. The patients were retrospectively assigned into two groups on the basis of outcome. Group 1 (n = 20) included infants who either had normal postnatal renal function or absence of prenatal renal dysplasia. Group 2 included infants who either had histologic evidence of renal dysplasia or subsequently developed renal failure. In group 1 the urinary sodium decreased and creatinine increased with gestation, demonstrating maturation in fetal renal function. In group 2 the urinary sodium and calcium were higher and the urinary urea and creatinine were lower than in group 1. The best predictor of outcome was the combination of either high calcium or high sodium with a positive predictive value of 91.3% and negative predictive value of 77.7%. In the antenatal evaluation of obstructive uropathy, fetal urinary biochemistry provides useful information for more accurate counseling of the parents and a rational basis for selecting patients who may benefit from intrauterine therapeutic interventions. (Am J Obstet Gynecol 1992;166:932-7.)

Key words: Fetal urine, fetal obstructive uropathy, urodochocentesis, pyelocentesis, ultrasonographic diagnosis

Prenatally, several parameters have been used in the evaluation of renal function in fetuses with obstructive uropathy, including amniotic fluid volume, ultrasonographic appearance of kidneys, and biochemical composition of fetal urine. The latter provides the most accurate information, and poor renal function is inferred from the findings of a high urinary sodium level (>100 mmol/L) and low urinary urea (<6 mmol/L) and creatinine (>150 μmol/L) levels.[1, 2]

In a retrospective study of 40 fetuses with obstructive uropathy Crombleholme et al.[1] reported that a urine sodium level of >100 mmol/L was predictive of poor prognosis, whereas a sodium level of <100 mmol/L was associated with good renal function. Similarly, Grannum et al.[3] measured urinary sodium in 12 fetuses with obstructive uropathy and demonstrated that the four fetuses with levels <100 mmol/L had either normal neonatal renal function or no evidence of renal dysplasia, whereas the eight fetuses with levels >100 mmol/L had renal dysplasia.

In contrast, Wilkins et al.[4] and Elder et al.[5] reported that fetal urine electrolytes are not accurate predictors of outcome. Thus in their combined series of 13 fetuses with obstructive uropathy only four of the seven with urinary sodium levels >100 mmol/L had poor outcome or histologic evidence of renal dysplasia, and only one of the six fetuses with levels <100 mmol/L had good renal function after birth.

One possible explanation for the apparent discrepancy in results is that in these studies no consideration was given for the gestation at which their fetuses were sampled; animal studies have demonstrated that the composition of fetal urine changes with gestation.[6, 7]

The aim of this study was to establish reference ranges with gestation for fetal urine sodium, calcium, urea, and creatinine and to examine the value of these substances in predicting the outcome of 60 fetuses with obstructive uropathy.

Patients and methods

In 60 fetuses with obstructive uropathy, urine samples were obtained by "urodochocentesis" (n = 52) or pyelocentesis (n = 8) at 16 to 36 weeks' gestation and sodium, total calcium, urea, and creatinine levels were measured on a multichannel, continuous-flow analyzer (SMAC II, Technicon, Basingstoke, United Kingdom). Urodochocentesis and pyelocentesis involve the ultrasonographically guided transabdominal insertion of a 20-gauge needle into the fetal bladder or renal pelvis, respectively, and the aspiration of urine. Urodochocentesis was performed when the fetal bladder was dilated and the ultrasonographic appearance of the two kidneys was similar. Pyelocentesis was performed when there was unilateral pelvicaliceal dilatation. In all cases fetal blood sampling and cytogenetic analysis demon-

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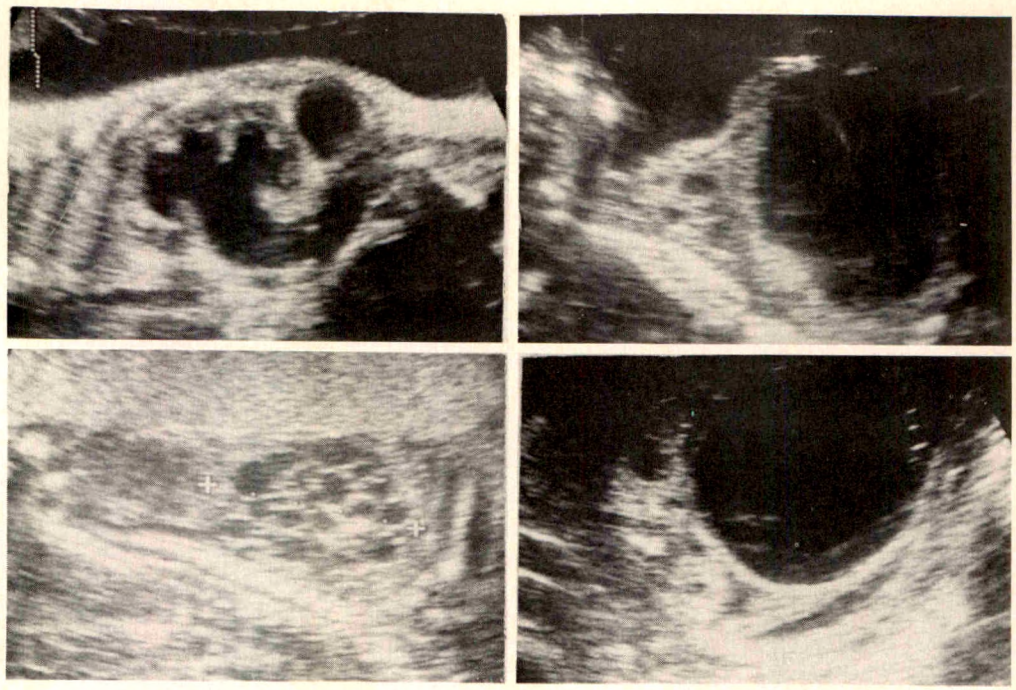


Fig. 1. Ultrasonographic appearance of fetal hydronephrosis with moderate-to-severe pelvicaliceal dilatation *(top left)*, multicystic dysplasia *(bottom left)*, and mild hydronephrosis with hyperechogenic cortex; longitudinal section shows dilated bladder *(top right)* and transverse section shows dilated bladder and echogenic kidneys *(bottom right)*.

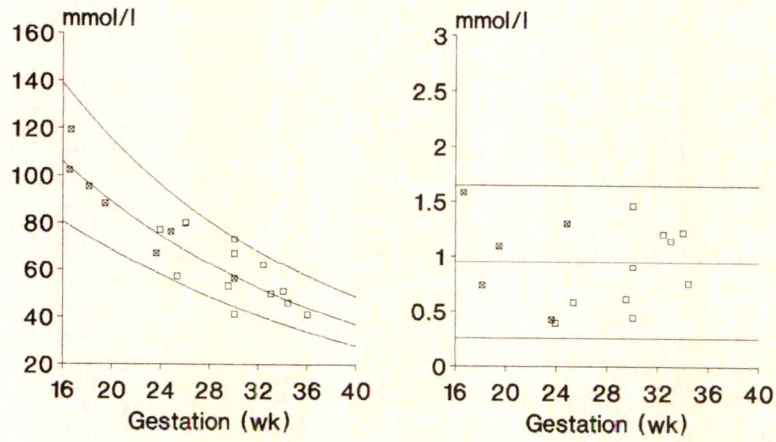


Fig. 2. Reference ranges (mean, 95th and 5th percentiles) with gestation for urinary sodium *(left)* and calcium *(right)* concentrations, calculated from study of fetuses with obstructive uropathy who either survived with normal postnatal renal function (□) or died but had no histologic evidence of renal dysplasia (☒).

strated a normal karyotype. The current study does not include data from fetuses who underwent vesicoamniotic shunting because this may have influenced the natural history of the disease. Similarly, the data from chromosomally abnormal fetuses that were sampled during the same period (1984 through 1990) are excluded.

Ultrasonographically the fetal kidneys were subdivided into three groups: (1) hydronephrotic with moderate-to-severe pelvicaliceal dilatation, $n = 40$; (2) multicystic dysplastic with multiple noncommunicating cysts of variable size and irregular, hyperechogenic stroma, $n = 9$; (3) mildly hydronephrotic with hyperechogenic cortex, $n = 11$ (Fig. 1). The latter type is commonly found in the presence of a grossly dilated bladder, usually before 20 weeks; in those cases where vesicoamniotic shunting is performed, after 4 to 8 weeks these kidneys usually develop the typical irreg-

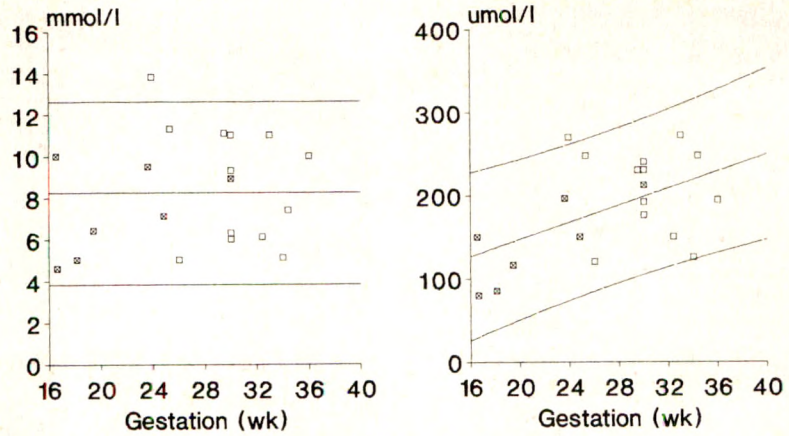


Fig. 3. Reference ranges (mean, 95th and 5th percentiles) with gestation for urinary urea *(left)* and creatinine *(right)* concentrations, calculated from study of fetuses with obstructive uropathy who either survived with normal postnatal renal function (□) or died but had no histologic evidence of renal dysplasia (☒).

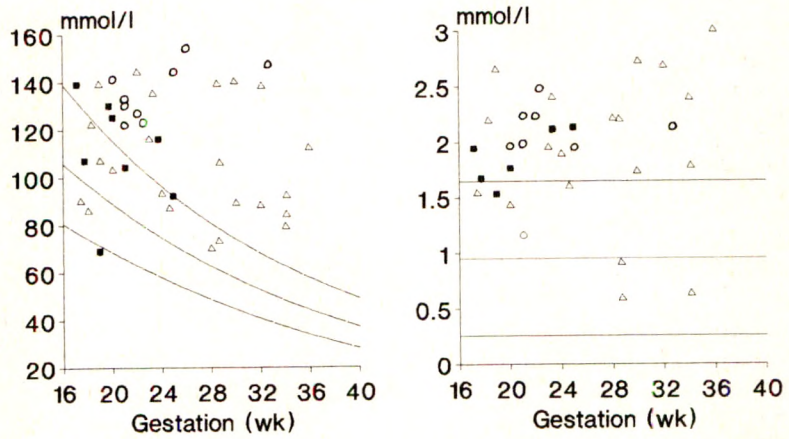


Fig. 4. Urinary sodium *(left)* and calcium *(right)* concentrations in fetuses with obstructive uropathy who either had histologic evidence of renal dysplasia or subsequently showed renal failure plotted on the appropriate reference range (mean, 95th and 5th percentiles) with gestation. Ultrasonographically, fetal kidneys were classified as hydronephrotic with moderate-to-severe pelvicaliceal dilatation (△), multicystic dysplastic (○), or mildly hydronephrotic with hyperechogenic cortex (●).

ular, shrunken, cystic, hyperechogenic appearances found in Potter stage IIb renal dysplasia (unpublished observations).

The 60 patients were retrospectively assigned into two groups on the basis of outcome. In group 1 (n = 20) 13 infants are alive with normal renal function (plasma creatinine concentration <70 μmol/L) at 1 to 6 (mean 2.1) years of age and seven fetuses were electively aborted (n = 6) or died in utero (n = 1) but had no histologic evidence of renal dysplasia. Group 2 (n = 40) includes (1) cases with histologic evidence of renal dysplasia (fetuses that were electively aborted, n = 22; fetuses that died in utero, n = 2; infants who died, n = 7; infants who are alive but required nephrectomy of the kidney sampled in utero, n = 4) and (2) infants who are in chronic renal failure at the age of 1 to 5 (mean 2.6) years (n = 5).

All patients were referred to our unit for diagnosis and further assessment. Subsequent management of the pregnancies was undertaken in the referring hospitals that provided the data on postmortem findings or follow-up of surviving infants.

Results

In group 1 there were significant associations between gestational age and fetal urinary sodium ($\log^{10}$ sodium concentration in millimoles per liter = 2.328 − 0.019 × gestational weeks, residual SD 0.062, r = 0.89, n = 20, $p < 0.0001$) and creatinine (creatinine concentration in micromoles per liter = 45.058 +

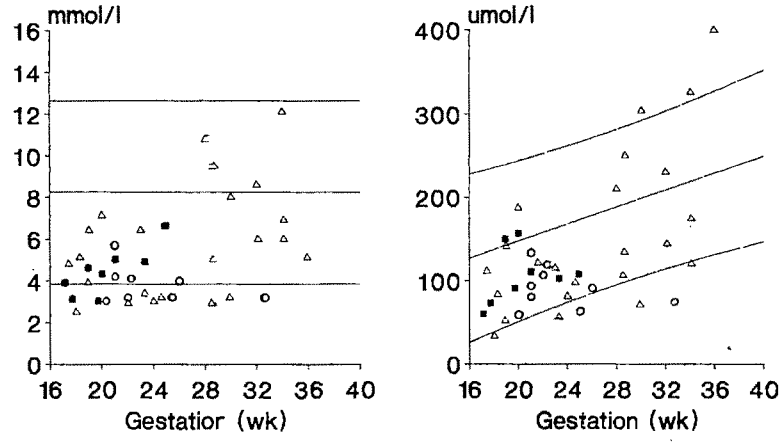


Fig. 5. Urinary urea *(left)* and creatinine *(right)* concentrations in fetuses with obstructive uropathy and either histologic evidence of renal dysplasia or subsequent development of renal failure plotted on appropriate reference ranges (mean, 95th and 5th percentiles) with gestation. Ultrasonographically, fetal kidneys were classified as hydronephrotic with moderate-to-severe pelvicaliceal dilatation (▲), multicystic dysplastic (○), or hydronephrotic with hyperechogenic cortex (●).

Table I. Incidence of oligohydramnios, high (>95th percentile) sodium or calcium levels, and low (<5th percentile) urea or creatinine levels in 60 cases of fetal obstructive uropathy grouped according to good or poor prognosis and subgrouped according to ultrasonographic appearances of fetal kidneys

	Group 1: Good prognosis			*Group 2: Poor prognosis*					
	Total	*Moderate-to-severe hydronephrosis*	*Mild hydronephrosis with hyperechogenic cortex*	*Total*	*Moderate-to-severe hydronephrosis*	*Mild hydronephrosis with hyperechogenic cortex*	*Multicystic dysplasia*	*Positive predictive value*	*Negative predictive value*
Oligohydramnios	5/20	4/17	1/3	25/40	13/23	6/8	8/9	77.8	52.2
Sodium >95th percentile	1/20	1/17	0/3	29/40	15/23	5/8	9/9	94.4	65.2
Calcium >95th percentile	0/17	0/15	0/2	25/33	13/19	5/6	7/8	95.0	71.4
Sodium/calcium >95th percentile	0/20	0/17	0/3	13/40	7/23	2/8	4/9	91.3	77.7
Urea <5th percentile	1/20	1/17	0/3	10/40	6/23	0/8	4/9	83.3	42.8
Creatinine <5th percentile	1/17	1/15	0/2	28/34	15/19	5/7	8/8	100.0	44.4

Positive and negative predictive values were calculated from the 41 cases in which the kidneys showed moderate-to-severe hydronephrosis or mild hydronephrosis with hyperechogenic cortex and all urinary substances were measured; cases of multicystic dysplasia were excluded because ultrasonographic appearances alone were always predictive of poor prognosis.

5.119 × gestational weeks, residual SD 52.453, $r = 0.52$, $n = 20$, $p < 0.05$) but not calcium (mean 0.935 mmol/L, residual SD 0.422) or urea (mean 8.245 mmol/L, residual SD 2.674). Reference ranges for these substances with gestation were established by regression analysis after it was ensured that the data were normally distributed (Figs. 2 and 3).

In group 2 the mean urinary sodium and calcium values were significantly higher ($t = 5.74$, $p < 0.0001$ and $t = 3.4$, $p < 0.01$, respectively) and the mean urinary urea and creatinine values ($t = 4.84$, $p < 0.0001$ and $t = -2.08$, $p < 0.05$, respectively) were significantly lower than in group 1 fetuses (Figs. 4 and 5).

The predictive value of the ultrasonographic appearance of the kidneys, oligohydramnios (no vertical pool of amniotic fluid ≥1 cm), and high (>95th percentile) sodium or calcium levels and low (<5th percentile) urea or creatinine levels are shown in Table I. All cases with multicystic kidneys had a bad outcome (renal dysplasia). However, 3 of the 9 (33%) fetuses with mildly hydronephrotic, hyperechogenic kidneys

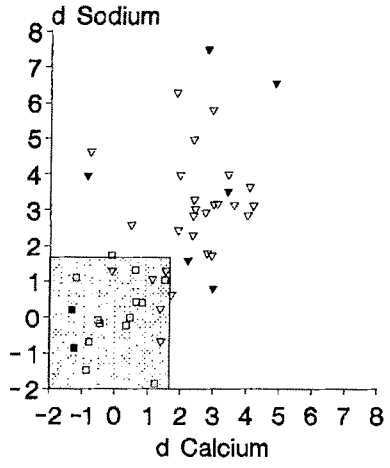


Fig. 6. Urinary sodium and calcium concentrations (expressed as numbers of standard deviations from appropriate normal mean for gestation) in fetuses with obstructive uropathy and either good (□) or poor (◇) prognosis. In 35 of 40 cases from poor prognosis group either sodium or calcium concentration was >95th percentile of reference ranges *(shaded areas)*. Biochemical findings of samples obtained from renal pelvis *(filled symbols)* are similar to those of samples from bladder.

and 17 of the 40 (43%) with moderate-to-severe hydronephrosis had a good outcome (no renal dysplasia or good renal function). In the latter two groups the best predictor (positive and negative predictive value) of outcome was the combination with either high calcium or high sodium (Fig. 6). There was no obvious difference in biochemical results of samples obtained by pyelocentesis and urodochocentesis.

Comment

This study establishes that in fetuses with obstructive uropathy and either normal postnatal renal function or absence of prenatal renal dysplasia the urinary sodium level decreases and the creatinine level increases with gestation, whereas values of calcium and urea do not change. In fetuses with obstructive uropathy and either histologic evidence of renal dysplasia or subsequent development of renal failure the urinary sodium and calcium levels are higher and the urinary urea and creatinine levels are lower than in the good prognosis group.

The concentration of urinary electrolytes and solutes in the good prognosis group, from which the reference ranges were established, is not necessarily representative of urinary biochemical findings in normal fetuses. However, establishment of normal ranges would be impossible. Furthermore, in the prenatal assessment of a fetus with obstructive uropathy it may be preferable to evaluate urinary biochemical levels in relation to reference ranges from fetuses with obstructive uropathy and good outcome rather than from normal fetuses.

Nevertheless, the number of cases used for construction of the reference ranges is small and the end points for inclusion are heterogeneous; lack of histologic evidence for intrauterine renal dysplasia in 7 of the 20 cases does not necessarily imply normal postnatal renal function. Furthermore, plasma creatinine levels of <70 μmol/L at 1 to 6 years does not guarantee that good renal function will persist. Indeed, in children with obstructive uropathy good renal function in the first few years of life is not necessarily predictive of good long-term outcome.[8] Therefore to establish more reliable reference ranges it will be necessary to undertake a multicenter study involving large numbers of fetuses with obstructive uropathy undergoing urodochocentesis, but not vesicoamniotic shunting, and surviving with normal renal function after long-term follow-up.

The urinary biochemical findings in the good prognosis group demonstrate a gestation-related maturation in fetal renal function. In normal urine production there is tubular reabsorption of sodium from an ultrafiltrate of serum. The fetal plasma sodium level is the same as that of the mother and does not change with gestation; therefore the observed decrease in urinary sodium concentration with gestation presumably reflects maturation in tubular reabsorption. Lind et al.[9] obtained fetal urine samples at hysterotomy for elective abortion at 12 to 22 weeks' gestation and demonstrated that as early as 12 weeks the kidney has considerable ability to resorb sodium. The high urinary sodium levels in fetuses with renal dysplasia are indicative of either tubular damage, presumably caused by the obstruction or primary maldevelopment of the kidneys, which is the most likely explanation for multicystic dysplasia.

Although calcium is also resorbed in the tubules, the fetal urinary concentration does not normally decrease with gestation. The most likely explanation for this finding is that increased tubular resorption with gestation is counterbalanced by the rising concentration of ionized calcium in fetal plasma and therefore in glomerular ultrafiltrate.[10, 11] Alternatively, the maximum resorptive capacity for calcium is fully established by 16 weeks' gestation because calcium is essential for fetal growth and development. The fetal hormone responsible for the strong conservation of calcium is probably parathyroid-related peptide, which is expressed in the fetal kidney as early as 12 weeks.[12] The high urinary calcium concentration in fetuses with renal dysplasia is likely a consequence of both impaired tubular resorption and proteinuria with loss of ionized and protein-bound fractions, respectively.

There is no significant renal resorption or secretion of creatinine, and the urinary concentration of this solute is merely a reflection of tubular resorption of water. The gestation-related increase in urinary creatinine concentration is likely a consequence of maturation of renal tubular function. In fetuses with renal dysplasia the urinary creatinine concentration is lower because

tubular resorption of water is impaired. However, a low creatinine level is not as sensitive a predictor of renal dysplasia as increased sodium or calcium levels. A possible explanation for this is that urinary creatinine concentration reflects not only tubular function but also the gestation-related increasing fetal plasma concentration.[13] This is dependent on muscle mass, which is unlikely to be affected by the degree of renal damage.

The urinary urea concentration does not change with gestation and is approximately three times higher than in fetal plasma.[9] This is unlike urinary sodium, which decreases because of active tubular resorption, or urinary creatinine, which increases because of active water resorption. In renal dysplasia electrolyte and water resorption are impaired, resulting in urinary urea concentrations that approach plasma levels.

In the antenatal evaluation of obstructive uropathy,the ultrasonographic finding of multicystic kidneys is associated with renal dysplasia; abnormal urinary biochemical findings merely confirm poor renal function. In contrast, in hydronephrosis both the degree of pelvicaliceal dilatation and the amniotic fluid volume are poor predictors of outcome; urodochocentesis or pyelocentesis with measurement of sodium, calcium, urea, and creatinine provides useful information for more accurate counseling of the parents. Furthermore, fetal urinary biochemical findings provide a rational basis for selecting patients who may benefit from vesicoamniotic shunting or other intrauterine urinary diversion procedures and allows evaluation of the possible effectiveness of such therapeutic interventions.

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Characteristics of three vaginal flora patterns assessed by Gram stain among pregnant women

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This study was undertaken to define the characteristics and persistence of vaginal flora in 7918 pregnant women at 23 to 26 weeks' gestation. Vaginal smears were categorized as normal (predominant lactobacilli), intermediate (reduced lactobacilli), or positive for bacterial vaginosis. The women with normal flora were least likely to have elevated vaginal pH, amine odor, milky discharge, or colonization by *Gardnerella, Bacteroides,* or genital mycoplasmas. Women with intermediate vaginal flora had intermediate frequencies of these clinical signs and microorganisms. Group B streptococci and yeast were associated with normal or intermediate flora, whereas *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were recovered more frequently from women with intermediate flora or bacterial vaginosis. *Trichomonas vaginalis* was most associated with intermediate flora. At follow-up, 81% of the women with normal flora had remained normal. Of the women with intermediate flora, 32% acquired bacterial vaginosis and 30% shifted to normal flora. Only 12% of the women with bacterial vaginosis had shifted to normal flora. We conclude that there are two primary stable vaginal flora patterns (normal flora or bacterial vaginosis) and a third less distinct transitional flora pattern between these two. (AM J OBSTET GYNECOL 1992;166:938-44.)

Key words: Bacterial vaginosis, vaginal flora

Pregnant women with the disturbed vaginal flora associated with bacterial vaginosis have an increased risk of amnionitis,[1] postpartum endometritis,[2] and preterm delivery,[3, 4] whereas women with *Lactobacillus*-predominant flora have a reduced risk of preterm delivery.[4] Accordingly, a simple, standardized laboratory test for evaluation of vaginal flora would be desirable. Direct microscopic evaluation of vaginal smears provides a rapid and inexpensive means of evaluating vaginal flora. First described by Dunkelberg[5] and later by Spiegel et al.,[6] the sensitivity of vaginal smears for diagnosis of bacterial vaginosis has ranged from 62% to 97% in three recent studies, with specificities of 66% to 95%.[7-9] The sensitivity and specificity of this method have varied, depending on the population studied and the evaluators of the smears. The low sensitivity or specificity for this test may be due to those smears that are neither *Lactobacillus* predominant nor typical of bacterial vaginosis but are forced into one or the other category because of the dichotomous nature of the criteria of Spiegel et al.[6]

Recently the intercenter reliability and reproducibility of recognizing various bacterial morphotypes in vaginal smears were evaluated.[10] The ability to diagnose bacterial vaginosis with the criteria of Spiegel et al. was only moderately reliable because of the wide variability in recognition of bacterial morphotypes by Gram smear evaluators.[10] A standardized scoring system for assessing the vaginal flora by Gram-stained smear was devised on the basis of the most reliably recognized morphotypes: large gram-positive rods (lactobacilli), small gram-negative to gram-variable rods (*Bacteroides* or *Gardnerella*), and curved gram-negative to gram-variable rods (*Mobiluncus*).[10, 11] The advantage of this objective scoring system is that it can accommodate flora patterns that are not typical of either normal flora or bacterial vaginosis.

To describe the association of genital microbiologic features and clinical signs with various vaginal flora patterns, we applied this standardized method in a large cohort of pregnant women. The epidemiologic characteristics associated with different patterns of vaginal flora and the persistence of these vaginal flora patterns during pregnancy are described.

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Material and methods

Patient population. Included in this study were 7918 women from five medical centers: Columbia Presbyterian Hospital, New York (2437 women); the University of Texas Health Science Center in San Antonio (1244 women); Louisiana State University, New Orleans (1460 women); the University of Washington, Seattle (611 women); and the University of Oklahoma Health Science Center, Oklahoma City (2166 women). To maximize consistency among the five study centers, all questionnaires, clinical examinations, and microbiologic procedures were performed according to a standardized protocol agreed on by the principal investigators at each center. In addition, one of the authors (R.P.N.) selectively observed the administration of questionnaires and the performance of clinical examinations at each site to optimize standardization. External quality control specimens were evaluated by the laboratories at each center for comparability, and all laboratories used commercially available reagents and media.

All women were enrolled during a routine prenatal visit at 23 and 26 completed weeks of gestation. A standardized medical, obstetric, sexual, and social history and cultures of the vagina and cervix were obtained. Women were excluded from the study for the following reasons: insulin-dependent diabetes mellitis, current use of corticosteroids or digitalis, chronic renal disease (serum creatinine level >2.5 mg/dl), symptomatic organic heart disease, plans to deliver at a nonstudy hospital, multiple gestation, prior use of tocolytic agents during the current pregnancy, cervical cerclage, hypertension requiring medication, age <16 years, Rh isoimmunization, or antibiotic use in the preceding 2 weeks. All women gave written informed consent approved by the respective institutional review boards of the participating centers.

Specimen collection. A clean, unlubricated speculum was placed into the vagina, and the appearance and pH of the vaginal discharge were noted. Sterile cotton swabs were used to obtain vaginal material from the posterior vaginal fornix for a vaginal smear. Prereduced sterile phosphate-buffered saline solution (3 ml) was placed into the vagina, and a vaginal wash suspension was prepared by swabbing discharge from the lateral vaginal walls. The suspension was then removed with a syringe and injected into an oxygen-free tube for transport. Discharge was wiped from the cervical portio, and the appearance of endocervical mucus and the cervix was noted.

Microbiologic methods. An endocervical swab for culture of *Neisseria gonorrhoeae* was used to inoculate Thayer-Martin and chocolate agar plates, which were incubated for 48 hours at 37° C in an atmosphere of 7% carbon dioxide. Cervical Dacron swabs for culture of *Chlamydia trachomatis* were transported in sucrose-phosphate buffer and inoculated onto cycloheximide-treated McCoy cells. *C. trachomatis* was identified within the infected monolayer by staining with fluorescent-labeled monoclonal antibodies.

A vaginal swab was used to inoculate Diamond's media for isolation of *Trichomonas vaginalis*, which was incubated at 37° C for 5 days in 7% carbon dioxide. Trichomonads were identified by visualizing motile forms in the wet mount of the broth under magnification ×400. Vaginal wash suspension (50 μl) was inoculated onto solid mycoplasma media and into broth media for the recovery of *Ureaplasma urealyticum* and *Mycoplasma hominis*. Genital mycoplasmas were identified by typical morphologic characteristics on solid media. Liquid media were examined daily for 7 days, and a color change indicating growth was confirmed by identifying typical colonies on subculture onto solid media.

Fifty microliters of the vaginal wash suspension was used to inoculate a sheep blood agar plate, human bilayer Tween agar, and a chocolate agar plate, which was incubated at 37° C in 5% to 7% carbon dioxide for 24 to 48 hours. Vaginal wash was also used to inoculate a selective broth for recovery of group B streptococci. For recovery of *Bacteroides* species, 50 μl of vaginal wash suspension was used to inoculate either a prereduced *Brucella* or Centers for Disease Control anaerobic blood agar plate and a laked blood kanamycin agar plate, which were incubated in either anaerobe jars (Oxford, Baltimore, or BBL, Cockeysville, Md.) or an anaerobic glovebox (Coy Laboratory Products, Ann Arbor) for 4 to 5 days at 37° C. *Gardnerella vaginalis* was identified by hemolysis on human blood agar, catalase reaction, typical Gram stain morphologic features, and lack of hemolysis on sheep blood agar. Facultative lactobacilli were identified by Gram stain morphologic features, catalase reaction, and colony morphologic features. Group B streptococci were typed with the Streptex latex agglutination typing system (Wellcome Diagnostics, Research Triangle Park, N.C.). Yeasts were identified by standard methods. *Bacteroides* spp. were identified by analysis of fatty acids by gas chromatography, the API A and An-Ident (Analytab products, Plainview, N.Y.), or the Minitek system (BBL Microbiology Systems). *Bacteroides* were identified only when present in high concentrations, i.e., growing in the third or fourth zone of the anaerobic agar plate. Because there was a lack of consistency between study centers with respect to the isolation of anaerobic gram-positive cocci, the data for peptostreptococci have not been included.

Criteria for evaluation of vaginal smears. Vaginal smears were air-dried, Gram stained, and evaluated under magnification ×1000. A score of 0 to 10 was

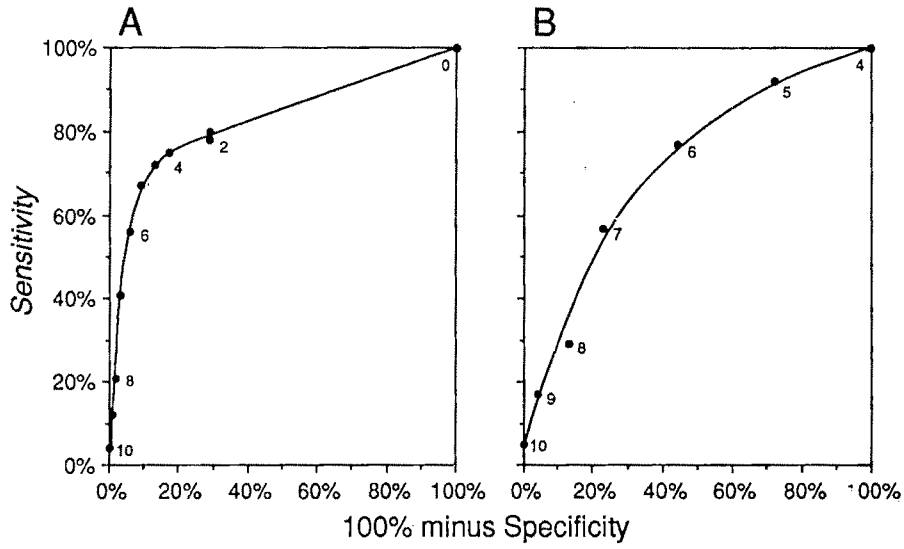


Fig. 1. Receiver-operator characteristic curves for bacterial vaginosis diagnosed by clinical signs and vaginal Gram smear score (0 to 10). Receiver-operator characteristic curves for scores of 0 to 10 **(A)** and 4 to 10 **(B)** suggest that there should be three divisions of the score at 0 to 3, 4 to 6, and 7 to 10.

assigned on the basis of the relative proportion of bacterial morphotypes (large gram-positive rods, small gram-negative or variable rods, or curved rods), with a score of 0 corresponding to the most *Lactobacillus*-predominant vaginal flora and a score of 10 corresponding to a vaginal flora characterized by replacement of lactobacilli by *Gardnerella, Bacteroides,* and *Mobiluncus* morphotypes. The vaginal Gram stain score was determined as follows: 4+ lactobacilli, 0; 3+ lactobacilli, 1; 2+ lactobacilli, 2; 1+ lactobacilli, 3; 0 lactobacilli, 4; 1+ *Gardnerella/Bacteroides,* 1; 2+ *Gardnerella/Bacteroides,* 2; 3+ *Gardnerella/Bacteroides,* 3; 4+ *Gardnerella/Bacteroides,* 4; 1+ to 2+ *Mobiluncus* spp., 1; 3 to 4+ *Mobiluncus* spp., 2.

Receiver-operator characteristic curves were formed by graphing the sensitivity versus 1 − specificity of the 0 to 10 score in predicting bacterial vaginosis diagnosed by three of four clinical signs.[12] The presence of three of four clinical signs (vaginal pH >4.5, amine odor after addition of 10% potassium hydroxide, the presence of clue cells, and a milky discharge) has been commonly used as the standard clinical diagnosis of bacterial vaginosis.[7] Because the wet mount examinations were not performed on all women, the association of the vaginal Gram smear score and the diagnosis of bacterial vaginosis with the standard clinical criteria was assessed in the subset of 711 women having clue cell examinations. To establish subcategories of the vaginal smear score, the association of the 0 to 10 score with the clinical signs of bacterial vaginosis was described by a receiver-operator characteristic curve.[12] The receiver-opertor characteristic curve discriminated between the frequency of bacterial vaginosis diagnosed by clinical signs among women who had a score from 0 to 3 (87% specificity) and women who had a score from 4 to 10 (72% sensitivity) (Fig. 1, *A*). A second receiver-operator characteristic curve was used to test the relationship of a score from 4 to 10 with the clinical signs of bacterial vaginosis (Fig. 1, *B*). Women with a score of 7 to 10 had a higher frequency of the clinical signs associated with bacterial vaginosis (78%) than women with a score of 4 to 6 (45%). These findings suggested three subdivisions of the 0 to 10 Gram stain score: normal, 0 to 3; intermediate, 4 to 6; and bacterial vaginosis, 7 to 10.

Analysis. Data were entered onto paper forms and mailed to the Data Coordinating Center (Research Triangle Institute, Research Triangle Park, N.C.) where the forms were entered into a mainframe computer. Data forms were edited and returned to the clinical centers for corrections where indicated. Data analysis was done with the Statistical Analysis System (SAS is the registered trademark of SAS Institute, Inc., Cary, N.C.).

χ^2 Tests were used to determine the statistical significance of the frequencies of genital isolates among the vaginal flora patterns.[13] The association of demographic characteristics with vaginal flora patterns were expressed by risk ratios and were assessed for statistical significance by 95% confidence intervals.[13] The adjustment of one demographic characteristic for another was performed by Mantel-Haenszel stratified analysis.[14]

Results

Of the 7918 women enrolled, 61% had vaginal smears consistent with normal flora, 16% intermediate flora, and 22% bacterial vaginosis. The proportion of women having elevated vaginal pH, amine odor, and milky discharge is shown in Fig. 2. The presence of a

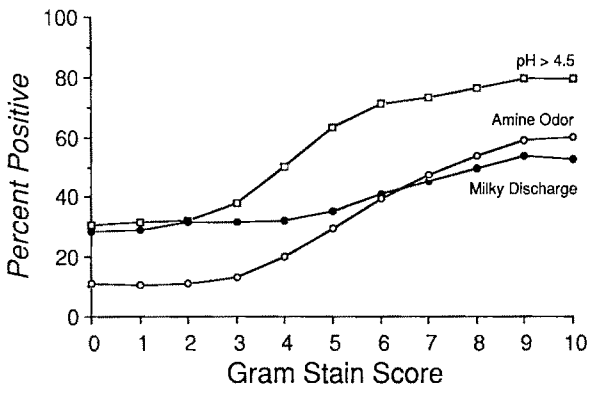


Fig. 2. Percent of women with positive results for milky vaginal discharge (●), amine odor after addition of 10% potassium hydroxide (○), and vaginal pH >4.5 (□).

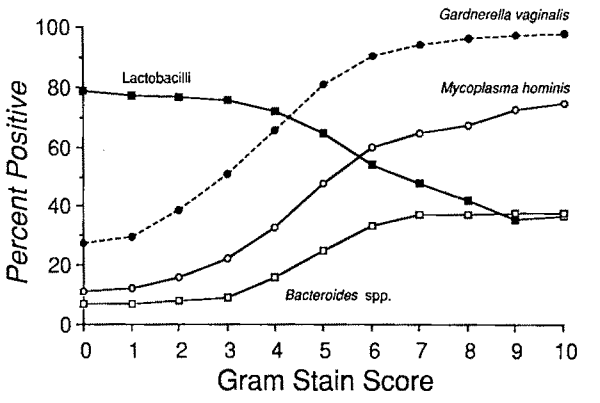


Fig. 3. Percent of women with positive results for facultative lactobacilli (■), *G. vaginalis* (●), *M. hominis* (○), and *Bacteroides* species (□).

Table I. Recovery of microorganisms from pregnant women with various vaginal flora patterns

	Gram smear interpretation		
	Normal	*Intermediate*	*Bacterial vaginosis*
G. vaginalis	1533/4867 (31%)	1040/1292 (80%)*	1692/1759 (96%)*
Lactobacillus†	3871/4863 (80%)	822/1294 (64%)*	773/1757 (44%)*
Bacteroides spp.†	355/4851 (7%)	324/1289 (25%)*	642/1751 (37%)*
Group B streptococci	928/4872 (19%)	244/1292 (19%)	261/1762 (15%)*
M. hominis	625/4835 (13%)	610/1282 (48%)*	1169/1748 (67%)*
U. urealyticum	3294/4816 (68%)	1018/1278 (80%)*	1492/1744 (86%)*
N. gonorrhoeae	35/4872 (0.7%)	25/1293 (1.9%)*	38/1717 (2.2%)*
C. trachomatis	308/4828 (6%)	155/1279 (12%)*	207/1732 (12%)*
T. vaginalis	441/4847 (9%)	275/1292 (21%)*	232/1749 (13%)*
Candida spp.	406/4398 (9%)	112/1294 (9%)	78/1760 (4%)*

*$p < 0.001$, compared with women with normal vaginal flora.
†Identified only when present in third or fourth streak zone of the agar plate.

milky discharge, a vaginal pH >4.5, and the release of amine odor after addition of 10% potassium hydroxide to the vaginal fluid was associated with an increasing vaginal Gram stain score. The data were similar at all five study centers.

The vaginal and cervical microbiologic characteristics stratified by vaginal flora patterns are presented in Table I. Since the vaginal score is based on a composite of *Lactobacillus, Gardnerella, Bacteroides,* and *Mobiluncus* morphotypes, it is not surprising that a strong association between these organisms and the vaginal flora patterns was observed (Fig. 3). The low frequency of *Bacteroides* species was not unexpected because only those isolates present at high concentrations (3+ to 4+) were reported. The degree of concordance between the smear interpretation and vaginal isolation suggests that these morphotypes can be recognized by direct examination of vaginal smears. *M. hominis,* an organism that cannot be observed in Gram-stained smears, was also recovered more frequently among women with intermediate flora or bacterial vaginosis (Fig. 3).

Group B streptococci were recovered from the vagina in 18% of the women. The recovery of group B streptococci was associated with a normal and intermediate vaginal flora (Table I). *U. urealyticum* was recovered less often from women with normal flora than from women with intermediate flora or bacterial vaginosis.

Women with normal vaginal flora were significantly less likely to be infected by *N. gonorrhoeae, C. trachomatis,* or *T. vaginalis* (Table I), suggesting an increased frequency of sexually transmitted disease pathogens in women with decreased lactobacilli (Fig. 4). The frequency of *N. gonorrhoeae* and *C. trachomatis* was similar for women with an intermediate or bacterial vaginosis flora pattern. However, *T. vaginalis* was most frequently recovered from women with intermediate vaginal flora (Fig. 4). *Candida* species were recovered from similar frequencies of women with normal and intermediate flora but were recovered significantly less frequently from women with bacterial vaginosis (Table I).

When compared with normal women, women with bacterial vaginosis were significantly more likely to be unmarried (relative risk 1.5, 95% confidence interval 1.4 to 1.7) and black (relative risk 1.7, 95% confidence interval 1.6 to 1.8) and less likely to have used an an-

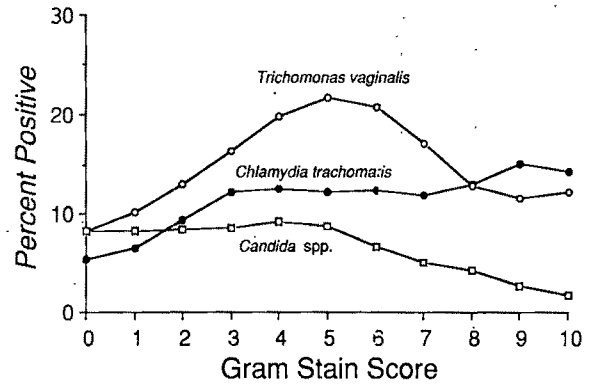


Fig. 4. Percent of women with positive results for *C. trachomatis* (●), *T. vaginalis* (○), and *Candida* species (□).

tibiotic before enrollment (relative risk 0.8, 95% confidence interval 0.7 to 0.9). The relationship between being black and having bacterial vaginosis was seen in four of the study centers. In New Orleans >85% of the women enrolled were black, so it was not possible to assess this relationship. The relationship between having bacterial vaginosis and being unmarried was statistically significant after adjustment for being black (risk ratio 1.3, 95% confidence interval 1.2 to 1.4). Bacterial vaginosis was not significantly related to a younger age at first intercourse, having had more than one sexual partner during the current pregnancy, more lifetime sexual partners, frequency of intercourse, smoking, drinking, gravidity, or parity (data not shown).

Women having an intermediate vaginal flora pattern were more likely than women with normal flora to be teenagers (relative risk 1.3, 95% confidence interval 1.2 to 1.5), to be black (relative risk 1.6, 95% confidence interval 1.4 to 1.7), to have had more than one sexual partner during the current pregnancy (relative risk 1.4, 95% confidence interval 1.1 to 1.6), and to have used antibiotics during the current pregnancy (relative risk 1.3, 95% confidence interval 1.2 to 1.4). The association between an intermediate vaginal flora pattern and these factors was consistent among all the study centers and was not altered if women with *T. vaginalis* or other sexually transmitted disease organisms were excluded from the analysis. Marital status, smoking, drinking, frequency of intercourse, gravidity, and parity were not associated with an intermediate flora pattern (data not shown).

Seven hundred sixty-two of the women were reevaluated at a return visit at 31 to 36 weeks' gestation. None of these women had used an antibiotic in the interim. Of the women with normal flora at enrollment, 81% were normal at follow-up (Table II). Of those having an intermediate flora pattern at enrollment, one third had acquired normal flora, one third had acquired bacterial vaginosis, and one third had persistent intermediate flora. Women with bacterial vaginosis flora at enrollment were most likely to have bacterial vaginosis at follow-up (69%), 19% had shifted to intermediate flora, and only 12% of the women shifted to a normal flora pattern.

Comment

The primary finding of the current study is that there are two major vaginal flora patterns and a third transitional vaginal flora, each having differing microbiologic and clinical characteristics. The concept that there are three vaginal flora patterns was first presented by Schroder[15] in 1921. He proposed that vaginal flora corresponded to the *reinheitsgrad* (degree of cleanliness) of the vagina. The first flora group (grade 1) was dominated by lactobacilli and considered to be the least pathogenic. The second group (grade 2) was characterized by mixed bacterial flora and a decrease in lactobacilli, whereas the third, and most pathogenic, group was composed of mixed flora without lactobacilli.[15] Siegler,[16] in 1946, reported that vaginal yeast and *T. vaginalis* were associated with grade 2 flora, whereas gonorrhea was associated with grade 3 flora. In the current study, with the use of standardized criteria for evaluation of the smears, vaginal *Candida* species were associated with normal and intermediate flora, and *C. trachomatis* and *N. gonorrhoeae* infection were more common in women with intermediate flora or bacterial vaginosis. *T. vaginalis,* which may be difficult to distinguish from bacterial vaginosis by clinical criteria, is most strongly associated with intermediate flora. The intermediate and bacterial vaginosis patterns are both associated with a decreased concentration and frequency of lactobacilli. It is not known whether these pathogens inhibit vaginal lactobacilli and thereby cause a shift in the vaginal flora, whether disturbed vaginal flora is a risk factor for acquisition of sexually transmitted disease, or whether there is an undescribed risk factor for both loss of lactobacilli and acquisition of sexually transmitted pathogens. Longitudinal studies will be required to address these issues.

The vaginal Gram stain score allows for a standardized interpretation on the basis of recognition of bacterial morphotypes. In the current study 7918 vaginal smears were interpreted at five centers by 14 different microbiologists. The ability to recognize selected bacterial morphotypes in the vaginal smears is supported by the culture data showing an increased frequency of *Gardnerella* and *Bacteroides* and a decreased frequency of facultative lactobacilli with an increased Gram stain score. *Mobiluncus* species, which are part of the score, were not routinely recovered from anaerobic vaginal cultures because only the anaerobes recovered in the

Table II. Persistence of vaginal flora patterns from enrollment to the third trimesters of pregnancy

Vaginal smear at enrollment (23-26 wk)	*Vaginal gram smear at follow-up (31-36 weeks' gestation)*			
	Total No.	*Normal*	*Intermediate*	*Bacterial vaginosis*
Normal	502	404 (81%)	61 (12%)	37 (7%)
Intermediate	119	36 (30%)	45 (38%)	38 (32%)
Bacterial vaginosis	141	17 (12%)	27 (19%)	97 (69%)

third and fourth streak zones of the culture plates were identified. The recovery of *M. hominis* was also associated with an increasing vaginal Gram stain score. The association of *M. hominis* with a diagnosis of bacterial vaginosis has been reported previously.[4, 9]

A vaginal flora pattern consistent with bacterial vaginosis was associated with being unmarried and black but not with having more lifetime sexual partners or more partners during this pregnancy, more episodes of sexual intercourse, smoking, drinking, or other medical or behavioral characteristics. The lack of association between lifetime sexual partners and bacterial vaginosis has been noted in another study.[17] However, an association between acquisition of bacterial vaginosis and exposure to a new sexual partner in the preceding month was detected in a longitudinal study.[18] It is possible that the cross-sectional nature of the study of Amsel et al.[17] and the current study would fail to detect a temporal relationship between a new sexual partner and acquisition of bacterial vaginosis.

Women with intermediate flora were more likely than women with normal flora to be teenagers, to be black, and to have had more than one sexual partner during pregnancy, even when women with sexually transmitted disease pathogens were excluded. Thus the epidemiologic characteristics associated with intermediate flora were not due to concomitant infection by sexually transmitted disease pathogens. These data suggest that the intermediate vaginal flora pattern may be a marker of increased sexual activity.

There are two stable types of vaginal flora, and a third, less distinct transitional phase between normal and bacterial vaginosis flora. The normal flora pattern appears to be the most stable, persisting in 81% of the women. Intermediate vaginal flora was the least stable, since 62% of the women had either switched to normal flora or acquired bacterial vaginosis between the two visits. Antibiotic use was significantly associated with having intermediate flora at enrollment, suggesting that some women have intermediate flora because of incomplete treatment of bacterial vaginosis or because of killing of lactobacilli by antibiotic therapy.

In the current study 69% of women with bacterial vaginosis in the second trimester of pregnancy also had bacterial vaginosis in the third trimester of pregnancy. Only 12% of the women had established normal flora. Bump et al.[19] have reported that 9 of 14 women had resolution of asymptomatic bacterial vaginosis without treatment in 6 months. Although our period of follow-up was shorter, our data suggest that bacterial vaginosis most often persists during pregnancy or shifts to intermediate rather than normal flora.

Infection by *N. gonorrhoeae,*[20] *C. trachomatis,*[3, 4, 21] and *T. vaginalis*[22] has been associated with adverse pregnancy outcomes. Bacterial vaginosis has been associated with amnionitis,[1, 2] preterm labor,[3, 4] preterm premature rupture of membranes,[4] and postpartum endometritis.[2] Microorganisms associated with bacterial vaginosis, including *M. hominis,*[21] *Bacteroides,*[23-25] and *G. vaginalis,*[25] have been associated with low birth weight or prematurity. Given the overlap between the vaginal flora patterns, these organisms, and infection by sexually transmitted pathogens, it will be of importance to evaluate whether bacterial vaginosis is asociated with prematurity or postpartum endometritis independent of these infections.

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Preterm premature rupture of membranes: Results of expectant management in patients with cervical cultures positive for group B streptococcus or *Neisseria gonorrhoeae*

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OBJECTIVE: The antepartum course and short-term neonatal outcome for patients with premature rupture of membranes between 26 and 33 weeks' gestation with positive cervical cultures for group B streptococcus or *Neisseria gonorrhoeae* were reviewed.
STUDY DESIGN: A retrospective analysis of 182 patients managed expectantly over a 3-year period was done. Thirty-four patients had cervical cultures positive for group B streptococcus, 11 had positive cultures for *Neisseria gonorrhoeae*, and 137 had negative cultures. Prophylactic antibiotics were routinely given, and antibiotic therapy was continued in patients with positive cultures.
RESULTS: There was no difference between groups in latent phase or maternal morbidity. The incidence of neonatal pneumonia was increased in those with positive cervical cultures (p = 0.009, odds ratio 6.9, 90% confidence interval 2.1 to 22.8), but there was no difference in neonatal sepsis, respiratory distress, or neonatal mortality.
CONCLUSION: These data support the conservative or expectant management of premature rupture of membranes between 26 and 33 weeks in patients with positive cervical cultures who are given prophylactic antibiotic therapy. (Am J Obstet Gynecol 1992;166:945-9.)

Key words: Premature rupture of membranes, group B streptococcus, *Neisseria gonorrhoeae*

Preterm premature rupture of membranes occurs in approximately 1% of all pregnancies.[1] Most women with this condition will eventually be delivered preterm, and the majority will do so within 7 days, irrespective of gestational age.[2] Infection with certain strains of bacteria may be an important cause;[3-5] an increased incidence of preterm premature rupture of membranes has been found among patients cervically colonized with group B streptococcus[6-8] or *Neisseria gonorrhoeae*.[9]

Although infection is implicated as a neonatal risk in patients with preterm premature rupture of membranes, the complications of prematurity, such as respiratory distress syndrome, are by far the more common causes of perinatal mortality.[10] Recent investigators have failed to show any significant association between preterm premature rupture of membranes[11-13] or a prolonged latent period[14, 15] and subsequent infection; the increased risk of neonatal infection has been primarily attributed to preterm delivery.[12] Expectant (conservative) management has been subsequently advocated to avoid the complications associated with preterm delivery.[11, 12, 14-18]

In this case-control study we sought to determine whether expectant management is justified in a subset of patients with preterm premature rupture of membranes who are at increased risk of maternal and fetal infection: those with positive cervical cultures for group B streptococcus or *Neisseria gonorrhoeae* at the time of membrane rupture. Of particular interest to the practicing obstetrician is the length of the latent period and the incidence of neonatal morbidity and mortality in those patients with positive cervical cultures and expectant management.

Material and methods

The maternal and fetal records were reviewed of all patients with preterm premature rupture of membranes occurring between 26 and 33 weeks' gestation who were admitted to the North Carolina Memorial Hospital obstetric service from July 1987 to September 1990. During this time there were 299 infants born to 275 women with this pregnancy complication. Pregnancies with preterm premature rupture of membranes at ≥34 weeks were not considered, because conservative management at this gestational age is not routinely practiced. Only those with singleton pregnancies in whom conservative management was planned were included in the analysis. Patients with premature rup-

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Table I. Comparison of demographics and maternal treatment characteristics in three study groups*

Parameter	Maternal cervical culture			Significance
	Positive group B streptococcus	*Positive gonorrhea*	*Negative*	
No.	34	11	137	
Maternal age (yr, mean ± SD)	25.6 ± 5.6	21.5 ± 4.1	25.1 ± 5.7	$p < 0.05$
Gestational age (wk, mean ± SD)	30.9 ± 2.0	29.8 ± 2.3	30.5 ± 2.2	NS
Parity	1.18	1.27	0.86	NS
Race				
White	12 (35%)	3 (27%)	63 (46%)	
Black	19 (56%)	7 (64%)	59 (43%)	
Other	3 (9%)	1 (9%)	15 (11%)	
Antibiotic use	32 (94%)	11 (100%)	114 (83%)	$p = 0.10$
Steroid use	14 (41%)	5 (45%)	46 (33%)	NS

NS, Not significant ($p > 0.10$). Borderline p values are given.

*The only difference noted was the younger age of those patients with premature rupture of membranes and cervical cultures positive for gonorrhea.

ture of membranes and labor, any sign of amnionitis on presentation, and those with other obstetric complications were not managed expectantly. Because we wanted to assess the effect of positive streptococcus and gonorrhea cultures on maternal and fetal outcome, patients with positive cultures for herpes simplex virus or chlamydia were excluded. Chlamydia cultures were not routinely done in patients with preterm premature rupture of membranes. Ninety-three women were excluded: labor on admission ($n = 41$), chorioamnionitis or fetal distress on admission ($n = 13$), multiple gestation ($n = 20$), herpes simplex virus genital tract colonization ($n = 3$), chlamydia cervical colonization ($n = 6$), known severe fetal anomaly ($n = 1$), placenta previa or severe preeclampsia necessitating delivery ($n = 4$), and records with insufficient documentation ($n = 5$). After these exclusions were made, 182 mother-infant pairs were included in the case-control analysis.

The diagnosis of rupture of membranes was made by history and by positive microscopic ferning and pH testing performed during speculum examination. Digital examinations generally were deferred until the onset of labor. Gestational age was determined by prenatal records, menstrual history, and ultrasonographic measurements made at admission.

At our hospital all patients with preterm premature rupture of membranes are admitted, cervical cultures for group B streptococcus and *N. gonorrhoeae* are routinely done, swab specimens are transported to the microbiology laboratory within 2 hours with Amies Charcoal Transport Medium. The microbiology laboratory bases the protocol for identification of these organisms on recommendations made by the American Society for Microbiology.[19, 20]

Prophylactic antibiotics (usually 1 gm intravenous ampicillin every 4 to 6 hours) were administered immediately and until the results of cervical cultures were known. If the culture for these two organisms was negative at 72 hours, antibiotic therapy was discontinued; in those with positive cultures, oral antibiotic therapy was continued for at least 7 to 10 days.

Steroids, to enhance fetal pulmonary maturity, were administered at the time of admission, at the discretion of each attending perinatologist. This was given as betamethasone, 12.5 mg intramuscularly, two doses, 24 hours apart. Sixty-five of the 182 patients received steriod therapy (Table I).

Expectant management of preterm premature rupture of membranes was practiced, and patients were closely monitored for signs or symptoms of chorioamnionitis. Monitoring consisted of frequent assessment of maternal temperature and uterine tenderness, daily electronic fetal monitoring, and daily or every-other-day determination of maternal white blood cell counts. Amniocentesis was not performed routinely, only if equivocal signs of amnionitis were present. Delivery was accomplished in all patients in whom the clinical diagnosis of chorioamnionitis was made. Tocolytics were not used in the 182 patients with premature rupture of membranes in this analysis.

Maternal outcome was assessed for the length of the latent period, defined as the interval between membrane rupture and active labor. Also assessed were the reason for delivery, the mode of delivery, and the incidence of postpartum endometritis. Neonates were evaluated for birth weight, Apgar scores, respiratory distress syndrome, sepsis confirmed by cultures, sepsis suspected but not proved, pneumonia, intraventricular hemorrhage, and length of neonatal intensive care stay.

Table II. Results of conservative management of preterm premature rupture of membranes: maternal outcome

Parameter	Cervical culture			Significance
	Positive group B streptococcus	*Positive gonorrhea*	*Negative*	
No.	34	11	137	
Latent period				
Mean ± SD (days)	4.7 ± 4.9	7.9 ± 7.9	4.3 ± 5.3	
Median (days)	3.0	2.8	2.6	NS
Range (days)	0.4-17.9	0.5-25.2	0.2-38	
Reason for delivery				$p = 0.06$
Amnionitis	9 (26%)	4 (36%)	30 (22%)	
Active labor	15 (44%)	4 (36%)	90 (66%)	
Fetal distress	6 (18%)	2 (18%)	14 (10%)	
Other	4 (12%)	1 (9%)	3 (2%)	
Cesarean section	6 (18%)	2 (18%)	26 (19%)	NS
Postpartum endometritis	6 (18%)	2 (18%)	29 (21%)	NS

NS, Not significant.

Respiratory distress was a clinical diagnosis, defined as tachypnea, with grunting, retraction, flaring, low oxygen saturation on room air, chest films showing reticulogranular, ground glass–appearing lungs with air bronchograms, and a negative evaluation for sepsis. Suspected sepsis was defined as signs or symptoms of sepsis in an infant with negative cultures, in whom antibiotic therapy was given for ≥3 days.

Statistical comparisons were made with the χ^2 test for discontinuous data and analysis of variance for group means. If data were not normally distributed, the Wilcoxon rank-sum test was used. Linear regression was used to determine the latent period as a function of gestational age. A p value of ≤0.05 was considered significant.

Results

Of 182 women with preterm premature rupture of membranes managed conservatively, the cervical culture obtained at admission was positive for group B streptococcus in 34 (19%) and for *N. gonorrhoeae* in 11 (6%); the cervical culture was negative in 137 (75%). These three groups of patients were compared demographically, as well as for gestational age, and for the use of steroid and prophylactic antibiotic therapy (Table I). The age of those patients with preterm premature rupture of membranes and positive cervical cultures for gonorrhea was significantly lower ($p < 0.05$) than the age of the other two groups. No other significant differences were found. It is important to note that there were no significant differences between the groups in the use of prophylactic antibiotic or steroid therapy.

The outcome of pregnancy, including the length of the latency period, reason for delivery, and cesarean delivery rate, was also compared in these groups, as shown in Table II. It is noteworthy that the latent period was not significantly affected by the presence of cervical colonization with these bacteria. There was no relationship between the length of the latent period and gestational age at premature rupture of membranes ($r = -0.17$, $p = 0.66$). Approximately 60% of the patients went into active labor after a variable period of expectant management. Amnionitis or fetal distress led to delivery in 65 women (36%). The reasons for delivery and the mode of delivery did not differ among the three groups. There was no difference in the incidence of postpartum fever between the three studied groups.

Table III summarizes the neonatal outcome in the three groups. There was no difference in mean birth weight or median time of stay in the intensive care nursery. Similarly, there was no difference in the incidence of proved or suspected sepsis or neonatal death.

There was a significantly increased incidence of pneumonia among the infants of patients with positive cervical cultures (relative risk = 6.9, 90% confidence interval 2.1 to 22.8, $p = 0.009$). Only 5 of the 34 patients with positive streptococcus cultures were classified as having a heavy growth. The median latent period in these 5 infants was 4.2 days, and there were no neonatal deaths. Of the five infants born to mothers with heavy colonization, one had proved sepsis (from *Escherichia coli*) and pneumonia, and one had suspected sepsis.

In patients who received steroid therapy, there was a trend toward a reduction in neonatal respiratory distress syndrome ($p = 0.06$). The use of steroids was not associated with an increased incidence of proved or suspected neonatal sepsis, even in those patients with positive cervical cultures ($p = 0.35$). The small number of patients with positive cultures, however, precludes

Table III. Results of conservative management of preterm premature rupture of membranes: Fetal outcome

Parameter	Cervical culture			Significance
	Positive for group B streptococcus	*Positive for gonorrhea*	*Negative*	
No.	34	11	137	
Sepsis				
Confirmed	2 (6%)	1 (9%)	7 (5%)	NS
Confirmed or suspected	6 (18%)	2 (18%)	11 (8%)	NS
Pneumonia	5 (15%)	1 (9%)	3 (2%)	$p = 0.009$
Intraventricular hemorrhage	6 (18%)	1 (9%)	19 (14%)	NS
Respiratory distress syndrome	16 (47%)	6 (55%)	70 (51%)	NS
Neonatal deaths (corrected)	2 (6%)	1 (9%)	5 (4%)	NS
Apgar scores (mean ± SD)				
One min	7.1 ± 2.0	6.2 ± 1.6	6.3 ± 2.3	NS
Five min	8.4 ± 0.8	8.0 ± 0.6	7.0 ± 1.4	NS
Neonatal intensive care unit stay (days)				
Median	8.0	7.0	9.0	NS
Mean	27.4	15.9	17.2	
Range	0-225	0-44	0-196	
Birth weight (gm)	1751	1675	1677	NS

NS, Not significant. Infants born to mothers with positive cervical cultures were significantly more likely to have pneumonia. See Comment section for power analysis.

definitive conclusions about the effect of steroids on the incidence of neonatal sepsis.

Comment

The expectant management of patients with preterm premature rupture of membranes and group B streptococcus cervical colonization has been evaluated in one previous study by Newton and Clark.[7] These investigators studied 140 consecutive patients with preterm premature rupture of membranes, 16 of whom were colonized with streptococcus. Patients with positive cultures had earlier rupture of membranes and shorter latent periods (76.8 vs 138.5 hours) than did those with negative cultures.

Amnionitis and endometritis were more common in mothers colonized with streptococcus. Neonatal infections were also significantly elevated in the infants of these mothers. One important difference between the study of Newton and Clark[7] and the current study is the use of prophylactic antibiotic therapy; in the former study antibiotics were only given intrapartum to group B streptococcus carriers. It is most likely that our results differed from theirs because of differences in the use of prophylactic antibiotic therapy.

Our study excluded those patients with situations necessitating aggressive obstetric management (chorioamnionitis, active labor, etc.) before determination of any effects of cervical colonization per se on the length of latent phase. Of those excluded patients, 16 women had positive cultures for streptococcus. The inclusion of these patients may have lead to a shorter latent period being associated with colonization. The practical question that this study addresses is how to care for an asymptomatic patient with preterm premature rupture of membranes managed expectantly, when the cervical culture is positive. In this regard we believe that our study exclusions are justified.

This investigation has two shortcomings. First, the study is retrospective, and relies on identification of abnormalities by different practitioners over a 3-year period. Most, but not all, patients received prophylactic antibiotic therapy (Table I). Although we presently perform chlamydia cultures on all patients with preterm premature rupture of membranes, these were not routinely done during the entire time of this analysis, and this may have confounded our results. However, Newton and Clark[7] did control for the presence of chlamydia and mycoplasmas, and the presence or absence of these organisms did not affect their findings.

Second, our study involves the evaluation of a relatively small population of patients with preterm premature rupture of membranes and positive cervical cultures, which may lead to type II statistical errors, especially when conditions with a low incidence are being studied. An analysis of type II error on neonatal outcome parameters in Table III shows that for neonatal respiratory distress syndrome, the power was >80% to detect a 30% increase in incidence in the patients with positive cervical cultures. Conversely, we would have had to study at least 435 patients to exclude a twofold increase in sepsis and at least 550 patients to exclude a twofold increase in neonatal deaths in the group with positive cultures. Although fetal distress appeared to occur more commonly in those patients with positive

cervical cultures, the factor necessitating delivery was not significantly different between the groups. Again, the finding of no difference may reflect a type II statistical error, because of the small numbers studied.

Nevertheless, the current study does provide a larger patient population on which to base clinical management in patients with this pregnancy complication. In any patient with preterm premature rupture of membranes, the clinician must weigh the risk of prematurity with that of maternal and neonatal infection. The risk of fetal infection appears to be increased by the presence of these bacteria in the maternal genital tract. It remains to be demonstrated, however, whether immediate induction of labor would reduce this risk when positive culture results are found. Particularly in the fetus of earlier gestational age, the risks of prematurity are substantial, and expectant management of selected asymptomatic patients with preterm premature rupture of membranes and a positive cervical culture may be justified to decrease these risks.

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Comparison of transdermal and oral estrogen-progestin replacement therapy: Effects on serum lipids and lipoproteins

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OBJECTIVE: We attempted to ascertain whether transdermal postmenopausal estrogen-progestin therapy has the typical effects of oral therapy on serum lipoprotein risk markers for cardiovascular disease.

STUDY DESIGN: Sixty-one postmenopausal women were randomized to receive either transdermal continuous 17β-estradiol, 0.05 mg/day, with transdermal cyclic norethindrone acetate, 0.25 mg/day, or oral continuous conjugated equine estrogens, 0.625 mg/day, with oral cyclic *dl*-norgestrel, 0.15 mg/day. Twenty-nine untreated subjects served as controls. Lipoprotein profiles at 3 and 6 months were compared with baseline values by means of analysis of variance.

RESULTS: In the estrogen-alone phase both therapies reduced serum levels of total and low-density lipoprotein cholesterol; high-density lipoproteins were largely unchanged. Oral therapy increased triglycerides whereas this lipid fell with transdermal therapy. In the combined phase of the cycle both therapies reduced triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.

CONCLUSION: Transdermal and oral therapies had similar effects on lipoprotein cholesterol but different effects on triglycerides. (Am J Obstet Gynecol 1992;166:950-5.)

Key words: Estrogen, progestin, lipoproteins, cholesterol, triglycerides

In addition to preventing osteoporosis, postmenopausal estrogen replacement therapy reduces the incidence of coronary heart disease, the major long-term consequence of the menopause, by 50%.[1] Although other mechanisms may be operating, this protection is consistent with the ability of oral estrogens to reduce serum levels of low-density lipoprotein (LDL) and raise those of high-density lipoprotein (HDL).[2]

To prevent endometrial hyperplasia and carcinoma, administration of progestin is advisable when treating nonhysterectomized postmenopausal women with estrogen.[5] Because progestins can oppose the potentially protective effects of estrogen on LDL and HDL,[4] there is a need for an effective postmenopausal regimen that retains the favorable lipoprotein profile typical of unopposed estrogen users. Administration of progestin by nonoral routes may achieve this by minimizing the unphysiologically high hepatic bolus seen with oral therapy (the first-pass effect). Because the liver is a central organ in lipoprotein metabolism, such avoidance of the first-pass effect may reduce nonphysiologic effects on serum lipoproteins.

A transdermal system delivering both estradiol and norethindrone acetate has recently been developed.[5] This regimen provides good cycle control and endometrial protection and prevents osteoporosis.[6, 7] We have now evaluated the effects of this regimen on serum lipoprotein risk factors for cardiovascular disease, using conjugated equine estrogens, the most widely used oral estrogen, as a comparator. Levonorgestrel was used as the oral progestin because it is available in the United Kingdom in a combination calendar pack. We also studied an untreated group to control for temporal changes in serum lipoprotein levels.

Material and methods

Participants. We studied 90 healthy white women before and during transdermal therapy, oral therapy, or no therapy. Women attending the menopause clinic, King's College School of Medicine, for relief of menopausal symptoms were randomized into two treatment arms. Because of concerns of the ethical committee, we were not permitted to randomize women into a placebo arm and instead recruited volunteers not requiring treatment to serve as a reference group.

All women were postmenopausal (between 6 months and 5 years) or, in the case of hysterectomized women, between 6 months and 5 years from the onset of meno-

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Table I. Baseline characteristics of three groups of women

Variable	*Reference group*	*Transdermal therapy*	*Oral therapy*
No. of subjects	29	31	30
Age (yr, mean ± SD)	53.8 ± 2.8*	52.1 ± 3.6	51.6 ± 3.5
Height (cm, mean ± SD)	162 ± 5	164 ± 7	164 ± 6
Body weight (kg, mean ± SD)	62.2 ± 6.5	63.7 ± 6.8	62.5 ± 6.5
% of Ideal body weight	104.0 ± 9.0	104.7 ± 9.2	101.8 ± 9.0
Oral contraceptive users in past (%)	21†	65	57
Menopausal age (mo, median and range)‡	36 (11-60)	19 (6-65)	36 (8-72)
% Hysterectomized	31	23	20

*$p < 0.05$, versus oral therapy.
†$p < 0.01$, versus both therapies.
‡Patients with intact uterus only.

pausal symptoms. Postmenopausal status was confirmed by measurement of gonadotropin levels. None had undergone castration. All were within 20% of their ideal body weight (Metropolitan Life Tables, 1959). None were taking drugs known to affect lipid metabolism, and none had ever received gonadal steroid pellets (implants), or steroids by any other route within the preceding 6 months. None smoked >10 cigarettes per day or drank >300 gm alcohol per week.

Sixty-one symptomatic women seeking therapy were randomly allocated to receive oral or transdermal therapy, while 29 women were recruited to the reference group. Oral therapy consisted of continuous conjugated equine estrogens (0.625 mg/day) together with *dl*-norgestrel (0.15 mg/day for 12 of each 28 days), supplied as a calendar pack (Prempak C, Wyeth-Ayerst, Maidenhead, England). Transdermal therapy also consisted of 28-day treatment cycles. The pharmokinetics and release characteristics of these patches have been described elsewhere.[5,8] Patches were applied to the buttock or lower abdominal wall and changed twice weekly. For the first 14 days of the cycle, patients applied an estrogen-alone patch (Estraderm TTS 50, Ciba-Geigy, Basel) that delivered 17β-estradiol approximately 0.05 mg/day. For the second 14 days of the cycle they applied a combination patch (Estragest TTS, Ciba-Geigy) that delivered approximately 0.05 mg 17β-estradiol plus 0.25 mg norethindrone acetate per day.

Women attended the Wynn Institute metabolic ward before commencement of the study and then after a further 3 and 6 months. Fasting blood samples were taken at each visit. During month 3, women receiving treatment were studied both at the end of the estrogen-alone phase (cycle 3A, days 12 to 14 with transdermal treatment or days 12 to 15 with oral treatment) and at the end of the combined phase (cycle 3B, days 26 to 28 with both therapies). At the 6-month visit, these women were studied only at the end of the combined phase (cycle 6). Compliance was monitored by serial measurement of gonadotropins and gonadal steroids, by counting used patches and pill packets, and by inspection of bleeding pattern charts completed by those women receiving treatment. Informed written consent was obtained before the study and approval was granted by the Ethical Committees of the Wynn Institute for Metabolic Research and King's College School of Medicine.

Laboratory analyses. Serum samples for lipid and lipoprotein assays were stored at 4° C and analyzed within 4 days; samples for other assays were stored at −20° C and analyzed within 6 months. Plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by immunoradiometric assay (MAIAClone, Serono Diagnostics, Surrey, England); plasma estradiol and estrone levels were measured as described previously.[9] Serum total cholesterol and triglycerides were measured by enzymatic assays. HDL and HDL subfraction 3 (HDL_3) cholesterol concentrations were measured after sequential precipitation with heparin and manganese ions[10] and dextran sulfate,[11] respectively. HDL subfraction 2 (HDL_2) cholesterol was calculated as the difference between HDL and HDL_3 cholesterol. Very-low-density lipoproteins (VLDL) were isolated by ultracentrifugation at solvent density 1.006 gm/ml.[12] LDL cholesterol was calculated as total cholesterol minus the cholesterol content of HDL and VLDL. Apolipoproteins AI, AII, and B were measured by immunoturbidimetry.[13] Coefficients of variation for these assays were 1% to 2% for total cholesterol and triglycerides, 2% to 3% for HDL, 5% to 7% for HDL_3, 6% to 8% for HDL_2, and 2% to 4% for apolipoproteins AI, AII, and B.

Statistical analyses. Data that were not normally distributed (estradiol, estrone, triglycerides, VLDL cholesterol, and VLDL triglycerides) were logarithmically transformed before statistical analysis. One-way analysis of variance, two-tailed unpaired Student *t* tests, Kruskal-Wallis one-way analysis of variance, Mann-Whitney U tests, and χ^2 analysis were used as ap-

Table II. Serum lipid and lipoprotein concentrations in untreated women and in subjects receiving either transdermal or oral estrogen replacement therapy for six cycles

	Total cholesterol (mg/dl, mean ± SD)	*Total triglycerides* (mg/dl, mean ± SD)*	*VLDL cholesterol* (mg/dl, mean ± SD)*	*VLDL triglycerides* (mg/dl, mean ± SD)*	*LDL cholesterol (mg/dl, mean ± SD)*
Reference					
Baseline	215 ± 25	80 (52, 123)	4.8 (2.0, 11.7)	23 (12, 45)	143 ± 27
Cycle 3	222 ± 26	76 (47, 123)	4.5 (1.4, 12.3)	21 (8, 55)	147 ± 29
Cycle 6	223 ± 26	78 (50, 119)	4.5 (1.8, 14.0)	20 (8, 50)	149 ± 33
Transdermal therapy					
Baseline	218 ± 32	70 (46, 105)	4.5 (1.8, 11.6)	19 (10, 39)	147 ± 29
Cycle 3A	204 ± 27†	58 (36, 91)	2.9 (1.4, 6.3)	13 (7, 26)	138 ± 27‡
Cycle 3B	198 ± 30†	56 (40, 80)	2.9 (1.4, 5.8)	12 (6, 25)	136 ± 31‡
Cycle 6	193 ± 32†	54 (37, 79)§	2.2 (1.1, 4.6)§	11 (5, 22)	131 ± 33†
Oral therapy					
Baseline	214 ± 36	69 (42, 111)	4.2 (1.5, 11.3)	17 (7, 41)	142 ± 36
Cycle 3A	195 ± 32†	79 (54, 115)§	4.2 (1.9, 9.1)	19 (8, 42)	123 ± 35†
Cycle 3B	188 ± 31†	62 (38, 99)	3.0 (1.2, 7.2)	14 (6, 32)	122 ± 35†
Cycle 6	184 ± 30†	60 (42, 86)	2.9 (1.4, 6.1)	12 (6, 26)	119 ± 32†

During cycle 3, subjects receiving therapy were studied while taking estrogen-alone and combined therapy (cycles 3A and 3B, respectively). During cycle 6, subjects receiving therapy were studied while taking combined therapy.

*Derived from logarithmically transformed data. *Values in parentheses* represent mean − SD and mean + SD, respectively.

†$p < 0.001$, indicating significant change from baseline value compared with change seen in untreated subjects.

‡$p < 0.01$, indicating significant change from baseline value compared with change seen in untreated subjects.

§$p < 0.05$, indicating significant change from baseline value compared with change seen in untreated subjects.

Table III. Serum apolipoprotein concentrations in untreated women and in subjects receiving either transdermal or oral estrogen replacement therapy for six cycles

	Apolipoprotein B (mg/dl, mean ± SD)	*Apolipoprotein AI (mg/dl, mean ± SD)*	*Apolipoprotein AII (mg/dl, mean ± SD)*
Reference			
Baseline	79 ± 15	148 ± 24	42 ± 4
Cycle 3	81 ± 16	152 ± 23	43 ± 5
Cycle 6	76 ± 15	147 ± 23	41 ± 3
Transdermal therapy			
Baseline	75 ± 13	142 ± 19	40 ± 4
Cycle 3A	72 ± 11*	134 ± 16†	37 ± 4†
Cycle 3B	69 ± 12†	128 ± 17†	33 ± 4†
Cycle 6	69 ± 13	130 ± 17*	37 ± 4‡
Oral therapy			
Baseline	73 ± 17	142 ± 21	41 ± 5
Cycle 3A	67 ± 16†	147 ± 17	42 ± 4
Cycle 3B	67 ± 16†	135 ± 16*	41 ± 4
Cycle 6	65 ± 14	136 ± 21	40 ± 5

During cycle 3, subjects receiving therapy were studied while taking estrogen-alone and combined therapy (cycles 3A and 3B, respectively). During cycle 6, subjects receiving therapy were studied while taking combined therapy.

*$p < 0.01$, indicating significant change from baseline value compared with change seen in untreated subjects.

†$p < 0.001$, indicating significant change from baseline value compared with change seen in untreated subjects.

‡$p < 0.05$, indicating significant change from baseline value compared with change seen in untreated subjects.

propriate to compare differences between patient groups, and two-tailed paired Student *t* tests were used to compare changes at each visit with baseline values.

Results

Three of the 61 women receiving treatment withdrew during the study. Two women receiving oral therapy complained of side effects (lower abdominal pain or breast tenderness and migraine) during the combined phase of therapy. One woman receiving transdermal therapy withdrew after experiencing pelvic pain similar to that diagnosed premenopausally as endometriosis. All 29 untreated women completed the study.

Baseline demographic data are shown in Table I. The reference group was slightly older than the oral therapy group, but there was no significant difference in menopausal age. There were no significant baseline differences in blood pressure, parity, alcohol intake, smoking habits, or reported exercise. The reference group had less previous exposure to oral contraceptives ($p < 0.01$). There were no significant changes in body weight during the study.

Both active treatments reduced plasma FSH and LH levels by approximately 50%. Although estradiol levels were increased by both therapies to a similar extent, estrone levels in the oral therapy group were twice as high as those in the transdermal group. Pretreatment estradiol levels in the reference group were marginally lower than those in the treated groups ($p < 0.05$). However, these levels increased at 3 and 6 months ($p < 0.05$), reaching the pretreatment levels seen in the treated groups. Estradiol levels in untreated women remained within the normal postmenopausal range throughout the study. One untreated subject experienced transient recurrence of spontaneous ovarian activity (estradiol level 566 pg/ml) at the 3-

HDL cholesterol (mg/dl, mean ± SD)	*HDL_2 cholesterol (mg/dl, mean ± SD)*	*HDL_3 cholesterol (mg/dl, mean ± SD)*
65 ± 15	23 ± 12	42 ± 7
66 ± 16	26 ± 12	41 ± 8
67 ± 17	25 ± 13	42 ± 7
65 ± 11	23 ± 8	43 ± 6
63 ± 11§	24 ± 8	39 ± 6§
59 ± 10†	21 ± 7*	38 ± 7§
59 ± 10‡	23 ± 7	37 ± 6†
66 ± 13	22 ± 10	44 ± 6
67 ± 12	23 ± 10	44 ± 7
61 ± 13†	21 ± 11‡	41 ± 5
62 ± 13‡	23 ± 10	40 ± 6†

month visit. Data from her 3-month visit were not analyzed.

There were no significant baseline differences in plasma lipids, lipoproteins, or apolipoproteins (Tables II and III). Both therapies lowered serum total cholesterol (Table II, Fig. 1), primarily because of decreases in LDL cholesterol. When the estrogen-alone phase (cycle 3A) was considered, oral therapy produced a significantly greater fall in LDL cholesterol than did transdermal therapy ($p < 0.05$); there were no significant differences between treatments during the combined phases of treatment (cycles 3B and 6). In both treated groups LDL cholesterol levels remained low when progestin was added (cycles 3B and 6). Declines in VLDL cholesterol (Table II) were seen in the transdermal group at all time points and in the oral group during the combined phases of treatment.

Oral treatment had no significant effect on HDL cholesterol during the estrogen-alone phase (cycle 3A), whereas transdermal therapy caused a minor (4%) fall in HDL cholesterol levels at this time (Table II, Fig. 1). During the combined phase (cycles 3B and 6), HDL cholesterol levels fell by 9% to 10% (transdermal therapy) or 5% to 7% (oral therapy). The two HDL subfractions responded differently to treatment (Table II). During the estrogen-alone phase of the treatments there was no significant change in HDL_2 cholesterol, but levels fell by 8% to 10% in both groups when progestin was added during cycle 3B, reverting to pretreatment levels during cycle 6. Levels of HDL_2 cholesterol increased by 14% in the reference group at cycle 3 ($p < 0.05$) but not at cycle 6. During the estrogen-alone phase of therapy, HDL_3 cholesterol fell in the transdermal group only, whereas levels of this lipoprotein were reduced by both therapies during the combined phase of treatment.

Changes in serum apolipoproteins (Table III) supported these observations about LDL and HDL cholesterol. Apolipoproteins B, the principal protein component of LDL, was lowered by both therapies during both phases of treatment, with no significant differences between treatments. During the estrogen-alone phase of treatment (cycle 3A), apolipoproteins AI, the principal protein component of HDL, was lowered by 5% by transdermal therapy ($p < 0.01$) but was unchanged by oral therapy. During the combined phase of treatment (cycles 3B and 6), apolipoproteins AI levels declined by 8% to 10% in the transdermal group ($p < 0.001$) and 4% to 5% in the oral group ($p < 0.05$). The between-treatment difference was significant during cycle 3A ($p < 0.05$) but not during the combined phases. Levels of apolipoproteins AII, a minor protein of HDL predominantly found in the HDL_3 fraction, was unchanged by oral treatment but lowered by 7% to 17% in women receiving transdermal treatment ($p < 0.001$). This between-treatment difference was statistically significant ($p < 0.01$) at all times.

In the estrogen-alone phase (cycle 3A), serum triglyceride levels were increased by 15% with oral therapy but reduced by 18% with transdermal therapy ($p < 0.05$) (Table II, Fig. 1). During the combined phases (cycles 3B and 6), triglyceride levels were reduced by 20% to 25% in both treatment groups. These differences between treatments were reflected in changes in VLDL triglyceride (Table II).

Comment

Both transdermal and oral therapies affected serum lipid and lipoprotein levels. Estrogens and progestins thus have effects on lipoprotein metabolism independent of any hepatic first-pass metabolism. Our findings show that administration of oral progestin, at a dose necessary to prevent proliferative endometrial transformation, does not negate the ability of estrogen replacement therapy to lower levels of LDL, a major cardiovascular risk factor. In addition, we have shown that LDL levels can be lowered substantially by transdermal estrogen replacement therapy and that transdermal progestin does not negate this effect. This would suggest that the effects of estrogen on LDL levels are largely physiologic, rather than representing pharmacologic actions on the liver.

Orally administered estrogen-progestin therapy may have profound effects on serum lipoprotein levels.[2, 14] We are unaware of any published prospective studies of transdermal combined therapy and serum lipoproteins. Reports of the metabolic effects of transdermal estrogen are conflicting. Although Chetkowski et al.[15] found no effects on LDL or HDL, this study was only of 1 month's duration. Another study[16] has shown an increase in HDL (but no change in LDL) with 0.1 mg/day transdermal estradiol, although this was in only 10 women.

The two therapies induced similar plasma estradiol levels. In spite of this, transdermal therapy had less

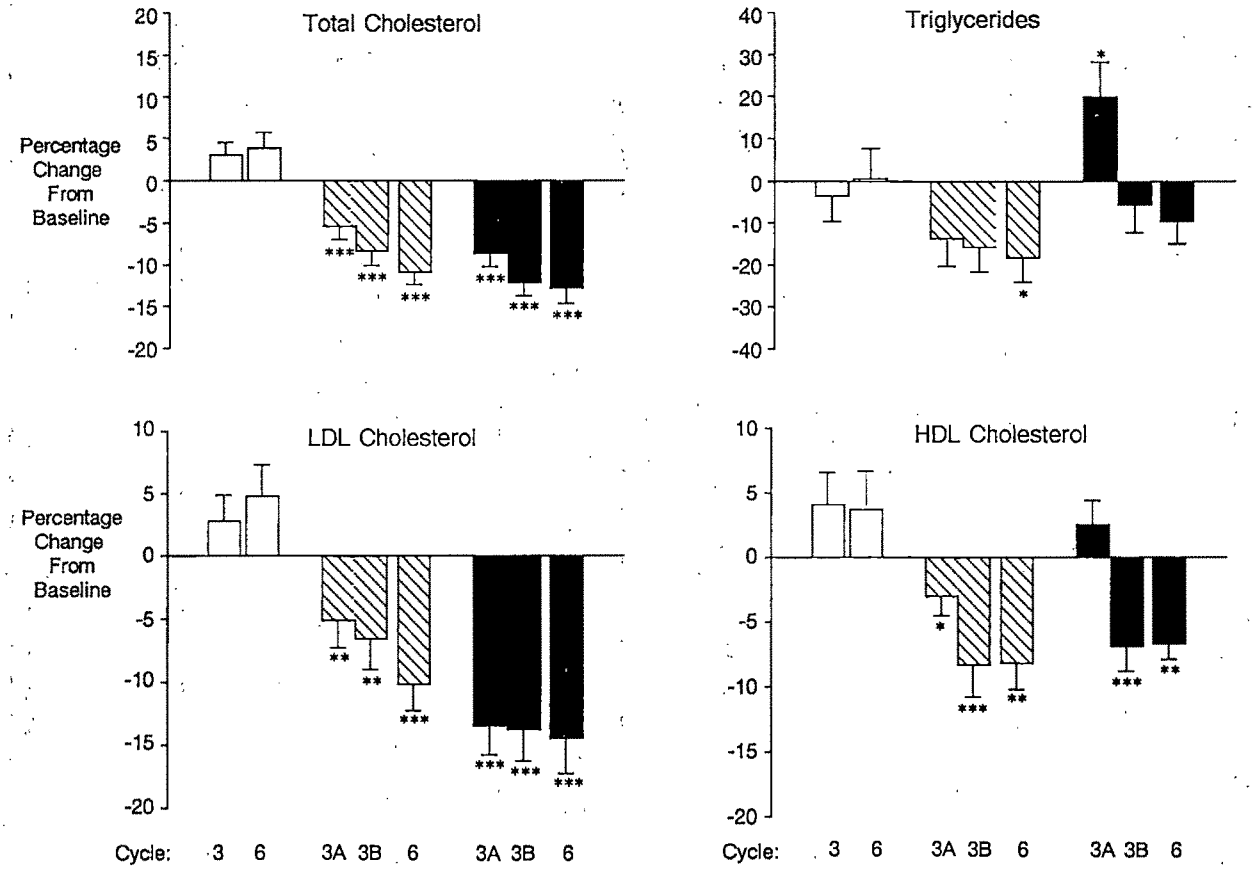


Fig. 1. Changes (mean ± SE) in serum lipids and lipoproteins in untreated subjects *(open bars)* and in subjects receiving either transdermal *(striped bars)* or oral *(solid bars)* estrogen replacement therapy for six cycles. During cycle 3, subjects receiving therapy were studied while taking estrogen alone and combined therapy (cycles 3A and 3B, respectively). During cycle 6, subjects receiving therapy were studied while taking combined therapy. *One asterisk,* $p < 0.05$; *two asterisks,* $p < 0.01$; *three asterisks,* $p < 0.001$; all indicate significant change from baseline value compared with change seen in untreated subjects.

effect on LDL levels than did oral therapy. This could be due to avoidance of the hepatic first-pass effect or to the relative potencies of individual components of the equine preparation. However, it could also be due to the lower plasma estrone levels seen in the transdermal group, because this hormone may lower LDL levels to a greater extent than does estradiol.[17]

We found no significant increase in HDL cholesterol levels in response to oral estrogen, although falls were seen when the progestin was added. This pattern of change was supported by changes in apolipoproteins AI, the major protein component of HDL. Although high doses of conjugated equine estrogens have been reported to increase levels of HDL, this effect is not always found when these steroids are given at a dose of 0.625 mg/day.[14, 18] Where an increase has been seen, addition of progestin may either have no effect, leaving HDL levels elevated, or may oppose the action of the estrogen and reduce HDL levels below baseline, depending on the androgenic characteristics of the progestin and on the balance of estrogen and progestin.[14, 18]

In the transdermal group, HDL levels fell during both combined and estrogen-alone phases, mainly because of falls in HDL_3. Apolipoproteins AII, a minor component of HDL distributed predominantly in HDL_3, was decreased only in the transdermal group, indicating that the fall in HDL_3 was associated with compositional changes in this fraction. Clearly, transdermal administration does not prevent norethindrone from lowering HDL, an observation supporting previous findings with vaginal administration of progestin.[19]

A limited number of studies, virtually all in men, indicate that the HDL_2 subfraction is responsible for the majority of the protective effect seen with HDL.[20] It is this fraction that is selectively increased by oral estrogen.[14, 18] Importantly, we found that the fall in HDL levels induced by both therapies was due primarily to a decrease in the HDL_3 subfraction, with no

consistent change in HDL_2. Such changes in HDL_3 may have a neutral impact on coronary heart disease risk. Interpretation of our HDL subfraction data is confounded by our finding of an increase in HDL_2 cholesterol in untreated women at cycle 3. However, the probability of obtaining a statistically significant change completely as a result of chance is high when a large number of biochemical measurements is performed in a study such as this.

Oral estrogen increased serum triglyceride levels, presumably by increasing the hepatic secretion of triglyceride-rich lipoproteins,[2] whereas oral combined therapy reduced these levels below baseline. A striking finding of the current study is that transdermal therapy lowered triglycerides during both estrogen-alone and combined phases. Although we cannot totally exclude a carryover effect of the progestin, a fall in triglycerides has been reported in women given unopposed percutaneous estradiol.[21] The physiologic effect of estradiol is to lower triglycerides, consistent with the lower triglyceride levels seen in premenopausal women compared with men,[22] and the increased triglyceride levels after ovariectomy.[23] The increase seen with oral estrogens is likely to be a pharmacologic effect, resulting from hepatic first pass. Thus transdermal estradiol appears to be more physiologic than oral estradiol in its effects on triglycerides. The ability of transdermal therapy to lower triglycerides may be important in view of growing evidence that this lipid is an independent risk factor for cardiovascular disease in women.[24]

In terms of cardiovascular risk, the optimal lipoprotein profile for an estrogen-progestin replacement therapy would combine elevated HDL (specifically HDL_2) levels with reduced LDL and reduced triglyceride levels. Our study shows that neither transdermal therapy (17β-estradiol and norethindrone acetate) nor oral therapy (conjugated equine estrogens and *dl*-norgestrel) gives such a profile.

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Oral contraceptive type and functional ovarian cysts

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OBJECTIVE: We tested the hypothesis that multiphasic, low-dose monophasic, and high-dose monophasic oral contraceptives share a common protective effect against functional ovarian cysts.
STUDY DESIGN: We conducted a cohort study using the automatic files of Maine Medicaid to assemble a population of 7462 women between the ages of 15 and 44 who were prescribed an oral contraceptive between Jan. 1, 1987, and Dec. 31, 1988. We included as cases 32 women with a principal diagnosis of a functional ovarian cyst confirmed by medical records as being >20 mm in diameter.
RESULTS: At comparison with the absence of an oral contraceptive prescription, we observed decreasing rates of functional ovarian cysts among women prescribed multiphasic pills (rate ratio 0.91, 95% confidence interval 0.3000 to 2.31), low-dose monophasic pills with ≤35 μg estrogen (rate ratio 0.52, 95% confidence interval 0.17 to 1.33), and high-dose monophasic pills with >35 μg estrogen (rate ratio 0.24, 95% confidence interval 0.01 to 1.34).
CONCLUSIONS: The protective effect of oral contraceptives against functional ovarian cysts reported previously for high-dose monophasic pills may be attenuated with newer pills of lower hormonal potency.
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Key words: Oral contraceptives, functional ovarian cysts

Oral contraceptives were introduced in the United States in 1960, and the most popular formulations through the mid-1960s were combination pills containing progestin and >50 μg estrogen.[1, 2] Besides being remarkably effective at preventing pregnancy, these high-dose combination pills also conferred certain health benefits, such as reducing the risk of functional ovarian cysts.[3-5] Publicity arose concerning possible adverse effects, however, and 1967 saw the introduction of low-dose formulations containing ≤35 μg estrogen.[1] Among the unintended effects of the early oral contraceptives was an increased risk of thromboembolic disease, which epidemiologic studies suggested was related to hormonal dose.[6] Possibly as a result of mounting concern about adverse effects, the popularity of the high-dose pills declined through the 1970s.[1, 2] This trend continued into the 1980s with the introduction in 1984 of multiphasic pills, which not only lowered hormone doses but also varied the dosage over the 21-day medication period in an attempt to mimic more closely the natural hormone cycle.[1]

In 1987, Caillouette and Koehler[7] reported on seven women in their clinical practice who were diagnosed with functional ovarian cysts soon after starting to use a multiphasic contraceptive. The hypothesized relation between multiphasic pills and functional ovarian cysts has biologic plausibility because multiphasic pills may not suppress ovarian function to the same degree as pills with higher hormone doses.[8-10] Initial investigation by Grimes and Hughes[11] showed that national trends in sales of multiphasic oral contraceptives were not correlated with hospitalization rates for functional ovarian cysts. Such correlations are of limited use in investigating etiologic relations, however, and even though no relation was apparent, the investigators cautioned that "this indirect assessment can neither confirm nor refute the postulated association."[11]

To date, there have been no measurements of the incidence of functional ovarian cysts among women using multiphasic oral contraceptives. We report here the rate of functional ovarian cysts associated with use of multiphasic pills, low-dose monophasic pills, and high-dose monophasic formulations of oral contraceptives, and we contrast these rates with one another and with the rate of cysts associated with nonuse of oral contraceptives.

Material and methods

We used the automated files of Maine Medicaid to identify a cohort of 9461 women who were prescribed an oral contraceptive from Jan. 1, 1987, to Dec. 31, 1988. We used Medicaid claims data to exclude from the primary analyses women who were not of reproductive age, who had cancer or ovarian dysfunction, or who were given drugs that might interfere with hormone metabolism. These drugs included fertility drugs and hormones, antipsychotics, anticonvulsants, and antitubercular drugs. After exclusions, the study population comprised 7462 women between the ages of 15

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and 44 years who contributed person time to the analysis from Jan. 1, 1987, to Dec. 31, 1988, during periods of Medicaid eligibility and when they were not classified as pregnant. To ensure that pregnancy periods were excluded, we excluded periods beginning 36 weeks before until 8 weeks after a delivery and 24 weeks before until 8 weeks after an abortion.

We sought to ascertain first occurrences during the study period of symptomatic functional ovarian cysts >20 mm in diameter. We identified cases from Medicaid billing claims and medical records for ultrasonographic procedures, hospitalizations, and outpatient surgical procedures. We reviewed medical records for hospitalizations and for outpatient surgical procedures that listed an ovarian cyst as a primary or secondary Medicaid diagnosis, but we accepted as cases only those women whose cyst was indicated by the medical record as the primary diagnosis. A diagnostic ultrasonographic procedure is sometimes associated in Medicaid claims data with a presumptive diagnosis. For example, an ultrasonographic procedure performed to rule out ovarian cancer might list ovarian cancer as the billing diagnosis even though the primary diagnosis might be an ovarian cyst. To identify cases diagnosed by ultrasonography but not listed in the claims file, we reviewed radiologists' reports for any pelvic ultrasonographic procedure that had a claim date from 3 months before until 1 week after a medical claim with any diagnosis of functional ovarian cyst. Again, we accepted as cases only those women whose cyst was the principal diagnosis of the ultrasonographic procedure. We defined as cases therefore women with a principal diagnosis of a functional ovarian cyst >20 mm in diameter. Acquisition and initial processing of Medicaid claims data, elicitation of hospital and clinic participation, and medical record abstraction was conducted by Maine Health Information Center.

We computed incidence rates as the number of functional ovarian cysts diagnosed between Jan. 1, 1987, and Dec. 31, 1988, divided by the sum of the person time contributed by the study population over the same period. Oral contraceptives were grouped into four types: multiphasic pills, low-dose monophasic pills (≤35 μg estrogen), high-dose monophasic pills (>35 μg estrogen), and progestin-only pills. Person time was allocated to three temporal categories of drug use: active prescription, recent prescription, and no prescription for an oral contraceptive. Women were considered to have an active prescription beginning on the date of the prescription and continuing for the number of days implied by the amount of medication supplied. Both 21-day and 28-day prescriptions were treated identically as 1 month of medication. We classified person time as recent use during the 30-day period after an active prescription. Any eligible day that did not fall within an active or recent prescription was assigned to the category of no prescription. Each prescription initiated an active prescription classification that truncated the classification in effect at the time of the prescription.

We compared first the rate of functional ovarian cysts during an active prescription for each type of oral contraceptive with the rate during no prescription for an oral contraceptive. Then, to describe differences between pills, we computed effect estimates for each type of oral contraceptive, using multiphasic pills as the reference category. We considered as potential confounding variables age (15 to 24, 25 to 34, 35 to 44), a prescription for antibiotics and duration of oral contraceptive use (≤1 month, >1 to 3 months, >3 to 6 months, >6 months). In the computation of duration of continuous oral contraceptive use, a prescription was considered continuous if it occurred within 5 days after the end of the previous prescription. We assessed and controlled for confounding effects of these variables by standardizing the rate in the reference category according to the covariate distribution in the index (i.e., exposed) category.[12] We also fit regression models to the data under the hypothesis that the observed number of cases follows a Poisson distribution.[13] The results from the regression models were virtually identical to those obtained from the simpler standardized analyses. We measured effects using incidence rate ratios, and we quantified the precision of each rate ratio estimate by computing 95% confidence intervals according to the exact mid-p method.[14]

Results

The study population of 7462 women contributed 179,088 person months, from which we excluded 31,676 person months as pregnancy periods and 50,934 person months as periods of Medicaid ineligibility. We sought medical records for 169 women in the study population, and we obtained records for 155 women. We did not obtain records for 14 women either because we could not find a medical record corresponding to the Medicaid claim (n = 6) or because the claim was from a medical facility from which no records were available (n = 8). Medical records were unavailable for women whose records were located at facilities that were out-of-state (n = 2) or closed (n = 1) or the facilities (or their representatives) decided not to participate for religious (n = 3) or other (n = 2) reasons. Of the 155 women for whom we obtained records, we confirmed 32 women as cases and 113 women as noncases. In addition, we did not include as cases 10 women for whom the medical records contained insufficient information to classify one way or the other according to our case definition.

To assess prescription claims as an indicator of drug use, we computed effect estimates on pregnancy for each oral contraceptive category (Table I). After comparison with periods when no oral contraceptive was

Table I. Rate ratio estimates for pregnancy comparing each oral contraceptive category with no oral contraception

	*Rate ratio**	*95% Confidence interval*
No prescription	1.00	Reference category
Recent prescription	0.65	0.55-0.77
Active prescription	0.35	0.31-0.39
Progestin-only	0.44	0.11-1.77
Multiphasic	0.32	0.27-0.39
≤35 μg estrogen	0.36	0.32-0.42
>35 μg estrogen	0.34	0.27-0.43

*Rate ratios standardized to age distribution of index (i.e., "exposed") category.

Table II. Incidence rates of functional ovarian cysts by oral contraceptive category

	No. of cases	*Person months*	*Rate (×10,000)*
No prescription	19	42,733.0	4.4
Recent prescription	2	8,872.8	2.3
Active prescription	11	44,053.2	2.5
Progestin-only	0	219.6	0.0
Multiphasic	5	13,196.4	3.8
≤35 μg estrogen	5	22,251.6	2.2
>35 μg estrogen	1	8,385.6	1.2

prescribed, these data show an approximate 65% reduction in the pregnancy rate for women prescribed multiphasic, low-dose monophasic, and high-dose monophasic pills and a 56% reduction for women taking progestin-only pills. Pregnancy rates declined with increasing duration of use, and there was a 75% reduction in the pregnancy rate after 6 months of continuous oral contraceptive prescriptions.

Table II describes the distribution of cases, person time, and incidence rates of functional ovarian cysts according to oral contraceptive category. The distribution of person time was divided nearly evenly between an active prescription (46%) and no prescription (45%) for an oral contraceptive, with the remaining time classified as recent use of oral contraceptives. Among types of oral contraceptives, the distribution was similar to national market shares[2]: 50% low-dose monophasic pills, 30% multiphasic pills, 18% high-dose monophasic pills, and <0.5% progestin-only minipills. Owing to sparse data, the progestin-only minipill is omitted from subsequent analyses of cysts. We confirmed 32 cases of functional ovarian cysts, with 19 cases occurring during no oral contraceptive prescription, two cases during the recent prescription period, and 11 cases during an active prescription.

After adjustment for slight confounding effects of age, comparisons with no oral contraceptive prescription revealed a 9% reduction in the rate of cysts for women using multiphasic pills, a 48% reduction in the rate among women using low-dose monophasic pills, and a 76% reduction in the rate for users of high-dose monophasic pills (Table III). In consideration of the hypothesis that these successive reductions in risk correspond to pills with increasing ovarian suppression, we conducted a regression analysis that used the information in each category simultaneously. The regression line for the multiplicative trend in the rate of cysts across these oral contraceptive categories has a slope of 0.76 (p value = 0.10), indicating progressive decline in the incidence of ovarian cysts with increasing ovarian suppression.

Among oral contraceptive categories, we used multiphasic pills as the reference category because the category with the lowest incidence rate, high-dose monophasic pills, included only one case. Compared with the incidence rate among women prescribed multiphasic pills, the rate of cysts among women using low-dose monophasic pills is approximately 40% lower (rate ratio 0.60, 95% confidence interval 0.16 to 2.16), and the rate of cysts among women using high-dose monophasic pills is about 67% lower (rate ratio 0.33, 95% confidence interval 0.01 to 2.54). Equivalently, the rate of functional ovarian cysts among women prescribed multiphasic pills is about 1.7 times greater than the rate among women prescribed low-dose monophasic pills and three times greater than the rate among women prescribed high-dose monophasic pills.

We examined the relation between rates of ovarian cysts and duration of continuous oral contraceptive prescription (Table IV). These data are sparse but show that among women with an active prescription for any type of oral contraceptive the overall incidence of functional ovarian cysts declines with increasing duration of use. The trends are not identical, however, for each type of oral contraceptive. For both low-dose and high-dose monophasic pills, the rate of cysts decreases with each category of increasing duration. For high-dose pills, the only case occurred during the 2231 person months accumulated during the first month of use, whereas no cases occurred during the subsequent 6154 person months. This trend is consistent, however, only for the monophasic pills. The rate of cysts among women using multiphasic pills declines in the duration category of >1 month and ≤3 months, then increases slightly with increasing duration.

Comment

In this population of women prescribed oral contraceptives, the incidence rate of functional ovarian cysts was greater during periods when no oral contraceptive was prescribed than it was during an active prescription for either monophasic pills or multiphasic pills. For users of oral contraceptives, however, this preventive effect against functional ovarian cysts appears to be

Table III. Rate ratio estimates for functional ovarian cysts comparing each oral contraceptive category with no oral contraception

	*Rate ratio**	*95% Confidence interval*
No prescription	1.00	Reference category
Active prescription:		
Multiphasic	0.91	0.30-2.31
≤35 μg estrogen	0.52	0.17-1.33
>35 μg estrogen	0.24	0.01-1.34

*Rate ratios standardized to age distribution of index (i.e., "exposed") category.

Table IV. Incidence rates of functional ovarian cysts by oral contraceptive category and duration of exposure

Duration (mo)	*Oral contraceptive*	*No. of cases*	*Person months*	*Rate (× 10,000)*
≤1	Progestin-only	0	70.8	0.0
	Multiphasic	2	3,969.6	5.0
	≤35 μg estrogen	2	6,566.4	3.0
	>35 μg estrogen	1	2,230.8	4.5
	TOTAL	5	12,837.6	3.9
>1-3	Progestin-only	0	72.0	0.0
	Multiphasic	1	4,144.8	2.4
	≤35 μg estrogen	2	6,842.4	2.9
	>35 μg estrogen	0	2,482.8	0.0
	TOTAL	3	13,542.0	2.2
>3-6	Progestin-only	0	52.8	0.0
	Multiphasic	1	2,818.8	3.5
	≤35 μg estrogen	1	4,759.2	2.1
	>35 μg estrogen	0	1,861.2	0.0
	TOTAL	2	9,492.0	2.1
>6	Progestin-only	0	24.0	0.0
	Multiphasic	1	2,263.2	4.4
	≤35 μg estrogen	0	4,082.4	0.0
	>35 μg estrogen	0	1,809.6	0.0
	TOTAL	1	8,179.2	1.2

greater for monophasic pills—especially high-dose pills—than for multiphasic pills. Two limitations of this study are its small size in relation to the frequency of functional ovarian cysts and the possibility of selective diagnosis of cysts among users of multiphasic oral contraceptives.

Misclassification of oral contraceptive use probably resulted in some underestimation of the effects of oral contraceptives on functional ovarian cysts. Because the inaccuracy of prescription data as a marker of drug use should apply similarly to each oral contraceptive category and to cases and noncases alike, misclassification of this type would be expected to diminish any apparent association.[15]

To investigate the extent of misclassification of oral contraceptive use, we examined the intended effects of oral contraceptives in reducing pregnancy rates. Compared with no oral contraceptive prescription, the pregnancy rate was reduced by approximately 65% for women with prescriptions for multiphasic, low-dose monophasic, or high-dose monophasic pills and about 56% for women using progestin-only pills. In addition, there was a trend toward decreasing pregnancy rates with increasing duration of oral contraceptive use, so that the pregnancy rate declined by about 75% after 6 months of use. The similar efficacy of monophasic and multiphasic pills,[16] the lower efficacy of progestin-only pills,[16] and the trend of increasing efficacy with duration of use[17, 18] have been suggested previously but cannot account entirely for the apparent efficacy of oral contraceptives being less than expected. Although the contraceptive effect is apparent in these data, the relatively large number of pregnancies suggests that a lack of compliance resulted in substantial underestimation of oral contraceptive efficacy, perhaps by as much as 35%. Nevertheless, this form of misclassification would not cause the appearance of an association where none existed and would not explain the association between oral contraceptive type and functional ovarian cysts.

Another potential source of exposure misclassification derives from the possibility that oral contraceptives were prescribed to treat a functional ovarian cyst. We examined oral contraceptive histories of all exposed cases to determine if any women were first prescribed the oral contraceptive type within a few months of the diagnosis. We identified one such case who was prescribed high-dose monophasic pills shortly before being diagnosed with a functional ovarian cyst. If the cyst was suspected and treated before the diagnosis, then this case should have been classified as having no oral contraceptive prescription. The effect of this bias would be to overestimate the incidence rate of ovarian cysts in the category of high-dose monophasic pills. Thus therapeutic use of oral contraceptives to treat ovarian cysts would not explain high-dose monophasic pills being associated with the lowest rate of ovarian cysts.

Misclassification of disease status is another potential source of error in this study. In particular, functional ovarian cysts are difficult to diagnose, and there may be a large group of occult cases that does not come to medical attention. Moreover, suspicion about a possible relation between multiphasic pills and functional ovarian cysts[7] could have resulted in cases being diagnosed preferentially among users of multiphasic pills. We addressed this issue by attempting to ascertain only symptomatic cases. We note also that physicians performing diagnostic procedures such as ultrasonographic examinations would not necessarily be aware of a patient's oral contraceptive history. Although we cannot rule out

a diagnostic bias, two lines of evidence run counter to this hypothesis. First, a suspicion concerning ovarian cysts and multiphasic pills should not apply to low-dose monophasic pills, because there has been no suggestion of a relation between ovarian cysts and low-dose monophasic pills. Thus the finding of a higher rate of cysts among users of low-dose monophasic pills than among users of high-dose monophasic pills should not be due to diagnostic bias. Second, any diagnostic bias for users of multiphasic pills should exist as soon as multiphasic pills are prescribed and might be expected to diminish with continued contraceptive use as the susceptible population is diagnosed and switched from multiphasic pills to other forms of contraception. During the first month of use, however, the results show little difference between types of oral contraceptives in the rate of cysts (Table IV). After the first month of use, the rate of cysts declines for users of monophasic contraceptives. For users of multiphasic pills, however, the rate of cysts declines briefly, but then increases with increasing duration of use. Thus the difference between pill types in the risk of functional ovarian cysts is enhanced with increasing duration of use. Rather than indicating a diagnostic bias, these data suggest that, like the pregnancy data, categories of longer duration of oral contraceptive use provide a more accurate indication of pharmacologic effects.

We controlled by either restriction or analytic means for potential confounding effects of age and duration of oral contraceptive use, for a variety of drugs that might affect hormone metabolism, and for the effects of cancer and ovarian dysfunction. Smoking data were unavailable from Medicaid, and we did not control for a possible effect of smoking. Smoking has been reported to be associated with an increased risk of ovarian cysts.[19] The reported effect was small, however, with the rate of cysts among current smokers being 50% greater than the rate among never-smokers. This difference could account for the difference in the rate of cysts between high-dose and low-dose monophasic pills or between low-dose monophasic and multiphasic pills, but it could not account for the threefold difference between multiphasic and high-dose monophasic pills even if everyone using multiphasic pills smoked and no one using high-dose monophasic pills smoked. It is possible that the effects of smoking are greater than has been estimated, but it is also unlikely that there is a great disparity in the prevalence of smoking among users of different types of oral contraceptives.

One reason that we restricted the cohort to women using oral contraceptives and did not include a comparison group of nonusers was to reduce differences between comparison groups with regard to extraneous risk factors (i.e., confounding). Within the cohort of oral contraceptive users, we selected as the primary reference category periods of no prescription because this category provided the most statistically stable effect estimates. If the protective effect of oral contraceptives lasts for longer than 1 month, then the rate observed here for the category of no prescription underestimates the true risk in the absence of oral contraceptives. This source of error, combined with the likelihood that some women did not use their prescribed oral contraceptives or did not use them properly, would bias rate ratio estimates toward a finding of no association. Thus the small decline in the rate of functional ovarian cysts observed for women prescribed multiphasic pills compared with no oral contraceptive is likely to underestimate the protective effect of multiphasic pills. Nevertheless, the possibility that multiphasic pills increase the risk of cysts compared with no oral contraceptive cannot be dismissed.

This is only the first study to investigate the risk of functional ovarian cysts in relation to multiphasic and other types of oral contraceptives. In addition, functional ovarian cysts are uncommon, and the small number of cysts in this study renders the incidence rates unstable and the effect estimates imprecise. Consequently, any or all of the differences between contraceptive types in the rate of ovarian cysts could be due to chance. To the extent that the categories of oral contraceptives used in this analysis correspond to different levels of ovarian suppression, however, this possibility becomes increasingly remote. The issue is best addressed, of course, by additional studies.

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Plasma endothelin levels in preeclampsia: Elevation and correlation with uric acid levels and renal impairment

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OBJECTIVE: The purpose of this study was to determine if endothelin levels are elevated in women with preeclampsia and if these levels correlated with other laboratory features of disease severity.

STUDY DESIGN: Parameters were compared in four groups of women volunteers by means of analysis of variance: (1) 16 women with preeclamptic pregnancies, (2) 11 pregnant women without preeclampsia, of similar lengths of gestation, (3) six otherwise normal women with pregnancies at term or beyond (>38 weeks), and (4) 22 normotensive young women.

RESULTS: Endothelin levels were elevated in women with preeclampsia as compared with those of gestation-matched pregnant and nonpregnant controls (22.6 ± 2.0 vs 12.0 ± 1.0 vs 10.4 ± 1.3 pmol/L, $p < 0.005$, preeclampsia vs controls) and also were increased in late gestation (17.7 ± 2.0 pmol/L). Endothelin correlated positively with plasma levels of uric acid ($r = 0.698$, $p < 0.005$) and inversely with creatinine clearance ($r = -0.659$, $p < 0.05$).

CONCLUSION: Circulating endothelin levels are elevated in women with preeclampsia and correlate closely with serum uric acid levels and measures of renal dysfunction. These observations suggest that endothelin may contribute to renal vasoconstriction in preeclampsia. (Am J Obstet Gynecol 1992;166: 962-8.)

Key words: Endothelin, preeclampsia, uric acid

Endothelin is a potent endogenous vasoconstrictor peptide produced by endothelial cells.[1] It is thought to participate in the regulation of vascular tone, but its normal physiologic and pathophysiologic role is unknown. Endothelin infusion into animals increases vascular resistance and decreases renal perfusion and glomerular filtration.[2-5] High-affinity binding sites for endothelin have been found in the placenta, and high levels of endothelin have been noted in umbilical vessels and fetal blood.[6-9]

Prominent features of preeclampsia include hypertension and impaired renal function with decreased uric acid excretion and glomerular endothelial cell injury.[10, 11] The mediators of these changes remain uncertain. To determine if endothelin levels are elevated in women with preeclampsia and if these levels correlate with other clinical and laboratory features that have been previously associated with the severity of this disease, we studied normotensive women and women with normal and preeclamptic pregnancies. We report elevated endothelin levels in preeclampsia and a close association between these levels and measures of renal dysfunction.

Methods

We examined four groups of women volunteers: (1) 16 pregnant women with preeclampsia at 27 to 38 weeks' gestation (age 20 to 40 years), (2) 11 pregnant women at similar stages of gestation (27 to 38 weeks) without preeclampsia (age 22 to 38 years), (3) six otherwise normal women with pregnancies at term or beyond (>38 weeks), and (4) 22 normotensive, nonmedicated healthy nonpregnant women (age 21 to 42 years). There were no preeclamptic pregnancies >38 weeks of gestation. None of the women was in labor at the time of study. Medical history and physical examination were performed at the time of phlebotomy. Three of the preeclamptic women were receiving intravenous magnesium sulfate, but none of the subjects were on any other antihypertensive medication at the time of study. The criteria for the diagnosis of preeclampsia were the appearance during the last trimester of ele-

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vated blood pressure (≥130/90 mm Hg), edema, elevated plasma level of uric acid (>5.0 mg/dl), and proteinuria (≥1+ on dipstick, approximately 30 mg/dl). One additional patient was included with only trace proteinuria but who met all other criteria. At the time of entry into the study, all women had blood samples obtained for endothelin, atrial natriuretic peptide (ANP), plasma renin activity, aldosterone, creatinine, and uric acid, and urine protein was assessed by dipstick. In addition, 11 of the 16 preeclamptic women and eight of the 11 normal pregnant women had 24-hour urine collections for total protein excretion and creatinine clearance. The preeclamptic women also had evaluation of liver function tests, platelet count, prothrombin and partial thromboplastin times, albumin, and hematocrit.

All studies were approved by the Committee on Clinical Investigation, New Procedures and New Forms of Therapy at the Beth Israel Hospital.

Blood samples for endothelin and ANF were collected into prechilled test tubes containing disodium ethylenediaminetetraacetic acid (1.5 mg/ml) and aprotinin (Trasylol, 400 kallikrein inhibitor units per milliliter; FBA Pharmaceuticals, New York). Blood was immediately centrifuged at 4° C. One milliliter of plasma was used for endothelin assay and 3 ml was used for ANP assay. Immediately after separation, plasma was acidified (0.25 ml 2N hydrochloric acid per milliliter plasma), extracted over ODS-silica cartridges (Sep-pak C18, Waters Associates, Milford, Mass.), washed with a solution of trifluoroacetic acid, and eluted with acetonitrile. Eluates were then lyophilized and stored at −20° C until just before radioimmunoassay (RIA). RIA for α-human ANP was performed as previously described with reagents obtained from Amersham, United Kingdom.[12] The lower limit of assay detection was 15.4 pmol/L (5.1 pg/ml), and the intraassay and interassay coefficients of variation were 6.7% and 10.8%, respectively.

Samples for endothelin were reconstituted in 250 μl of 0.02 mol/L borate buffer just before assay. RIA was performed with a standard double-antibody precipitation technique with reagents obtained from Amersham. Antiserum was specific for endothelin-1 and endothelin-2, with 30% cross-reactivity with Big endothelin and without cross-reactivity with endothelin-3 or ANP. After addition of antibody and trace to 100 μl of sample, tubes were again incubated at 4° C. After 72 hours, goat antirabbit antiserum (dilution 1:8) (Pel-Freez, Rogers, Ark.) was added and incubation was continued an additional 24 hours. Bound from free radioactivity was then separated by centrifugation at 4° C for 30 minutes at 3200 rpm with subsequent decantation of the supernatant. Bound radioactivity was determined by counting each tube for 2 minutes in a γ-scintillation counter. The sensitivity as determined by 50% displacement of tracer was approximately 20 fmol per tube. The lower limit of assay detection was 1 fmol per tube. The intraassay and interassay coefficients of variation were 7% and 15%, respectively.

Plasma renin activity was determined by RIA of the generation of angiotensin I with kits purchased from Baxter, Cambridge, Mass. Serum aldosterone was determined with RIA kits purchased from Diagnostics Products Corporation, Los Angeles.

Creatinine determinations were performed in the clinical laboratory with a rate-dependent modification of the Jaffe reaction that minimizes the influence of slow-reacting chromogens (SMAC II, Technicon Instruments, Inc., Tarrytown, N.Y.).

All other laboratory evaluation was performed in the clinical chemistry and hematology laboratories at the Beth Israel Hospital.

Statistics. Power analysis that was based on preliminary endothelin measurements (with the β error set at 0.8 and the significance level at 0.05) suggested that, on average, seven subjects should be enrolled in each group. Statistical analysis was performed with one-way analysis of variance and determination of Pearson's correlation coefficients. Values were considered statistically significant at a p value of <0.05. All values are expressed as mean ± standard error.

Results

Subject characteristics (Table I). All but one of the preeclamptic women were primigravid; all of the controls were primigravid. Arterial blood pressures were highest in the preeclamptic group and lowest in the pregnant controls (mean arterial pressure 111.7 ± 2.5 vs 77 ± 1.5 vs 83.5 ± 2.3 mm Hg, systolic pressure 144 ± 3.5 vs 102 ± 3 vs 114 ± 2 mm Hg, diastolic pressure 95 ± 2.4 vs 64 ± 2 vs 70 ± 2; preeclampsia vs pregnant and nonpregnant controls $p < 0.05$). The mean duration of gestation was similar in both the women with preeclampsia and pregnant controls (34.3 ± 0.8 vs 32.8 ± 1.1 weeks). There were six twin gestations in the preeclamptic group and none in the pregnant control group. The mean duration of gestation in the preeclamptic twin pregnancies (33 ± 1.6 weeks) was not different from that in the singleton preeclamptic pregnancies (35 ± 0.9 weeks).

Clinical laboratory features (Table I). As anticipated, the preeclamptic group had higher uric acid levels than the normal pregnant women. They also had higher serum creatinine levels. The average 24-hour urine creatinine clearance was 103 ± 5 ml/min, and protein excretion was 1.67 ± 0.57 gm/day in the 11 preeclamptic patients in whom it was measured. The average 24-hour creatinine clearance and 24-hour protein excretion was normal in the eight normal pregnant women

Table I. Subject characteristics of preeclamptic group and normal pregnant subjects

	Preeclamptic	*Normal pregnant*
No.	16	11
Gestation (wk)	34.3 ± 0.8	32.8 ± 1.1
Age (yr)	30.6 ± 1.8	31.5 ± 1.5
Arterial blood pressure (mm Hg)	112 ± 2	77 ± 2*
Uric acid (mg/dl)	7.1 ± 0.3	3.8 ± 0.2*
Serum creatinine (mg/dl)	1.0 ± 0.03	0.74 ± 0.03*
Creatinine clearance (ml/min)	103 ± 5 (n = 11)	126 ± 6 (n = 8)*
Protein excretion (gm/24 hr)	1.67 ± 0.57	0.20 ± 0.05*
Hematocrit (%)	35 ± 1.5	35.4 ± 1.0 (n = 7)
Platelet count (cells × 10^3/mm^3)	184 ± 17	201 ± 15 (n = 7)

Values are mean ± SD. Subjects were similar in age and gestational duration but had significant differences in blood pressure, uric acid, serum creatinine, creatinine clearance, and protein excretion.

*$p < 0.05$.

Table II. Peptide and hormone levels in preeclampsia, normal pregnancy and nonpregnancy

	Preeclampsia	*Normal pregnancy*	*Nonpregnancy*
Endothelin (pmol/L)	22.6 ± 2.0*	12.0 ± 1.0	10.4 ± 0.4
ANP (pmol/L)	93 ± 36	55 ± 8	53 ± 3
Plasma renin activity [ng/(L · sec)]	3.4 ± 1.1†	2.8 ± 0.6†	0.7 ± 0.1
Aldosterone (pmol/L)	1395 ± 261‡	2665 ± 350†	652 ± 78

*$p < 0.005$, versus normal pregnancy and nonpregnancy.

†$p < 0.05$, versus nonpregnancy.

‡$p < 0.05$, versus normal pregnancy.

in whom it was measured. There was also a trend toward lower platelet counts in the preeclamptic patients, although this did not reach the level of statistical significance. Three of the patients with preeclampsia had elevated values on liver function tests.

Endothelin levels (Table II). Endothelin levels were highest in the women with preeclampsia, 22.6 ± 2.0 pmol/L ($p < 0.005$), as compared with the normal pregnant women (12.0 ± 1.0 pmol/L) and the nonpregnant controls (10.4 ± 0.4 pmol/L). The levels in the preeclamptic twin pregnancies (24.3 ± 3.6 pmol/L) were not different from those in the singleton preeclamptic pregnancies (21.6 ± 2.4 pmol/L). The levels in the normal pregnant women were not different from those of the nonpregnant women.

ANP, aldosterone, and renin levels (Table II). Although ANP levels tended to be highest in the preeclamptic group, levels were not significantly different in any of the groups (93 ± 36, 55 ± 8, and 53 ± 3 pmol/L in preeclamptic group, pregnant group, and nonpregnant controls, not significant). However, there was greater variability in ANP levels in the preeclamptic group: three were low (<20 pmol/L), nine were normal (20 to 90 pmol/L), and three were abnormally high (126, 412, and 474 pmol/L). One did not have ANP measured. In contrast, ANP levels in the normotensive pregnant women were normal in 10 (30 to 85 pmol/L) and slightly high in one (120 pmol/L). The range of ANP levels was 35 to 75 pmol/L in the nonpregnant women and 23 to 79 pmol/L in the late-gestation group.

Aldosterone levels in the preeclamptic group were significantly lower than those in the normal pregnant women, although still higher than those of the nonpregnant women.

Plasma renin activity was similarly elevated in both pregnant groups as compared with that in nonpregnant controls.

Correlation analysis (Fig. 1). In patients with preeclampsia but not in controls, endothelin levels were strongly positively correlated with uric acid levels ($r = 0.698$, $p < 0.005$, $y = 0.1257x + 4.49$). Endothelin was also positively correlated with serum creatinine ($r = 0.595$, $p < 0.05$, $y = 0.0136x + 0.668$) and inversely correlated with creatinine clearance ($r = -0.659$, $p < 0.05$, $y = -1.457x + 135$). There was no correlation with blood pressure or 24-hour protein excretion.

ANP levels were directly correlated with mean arterial blood pressure ($r = 0.571$, $p < 0.05$) in the preeclamptic group but not in the control groups. There was no significant correlation between ANP levels and endothelin, creatinine clearance, serum creatinine, serum albumin, hematocrit, aldosterone, plasma renin activity, or multiple gestation.

Influence of duration of gestation (Fig. 2). To determine if length of gestation influenced endothelin levels, six primigravid women with otherwise normal pregnancies but who were late in gestation (>38 weeks) were also evaluated. The mean duration of gestation in these women was 39.8 ± 0.5 weeks. The mean ar-

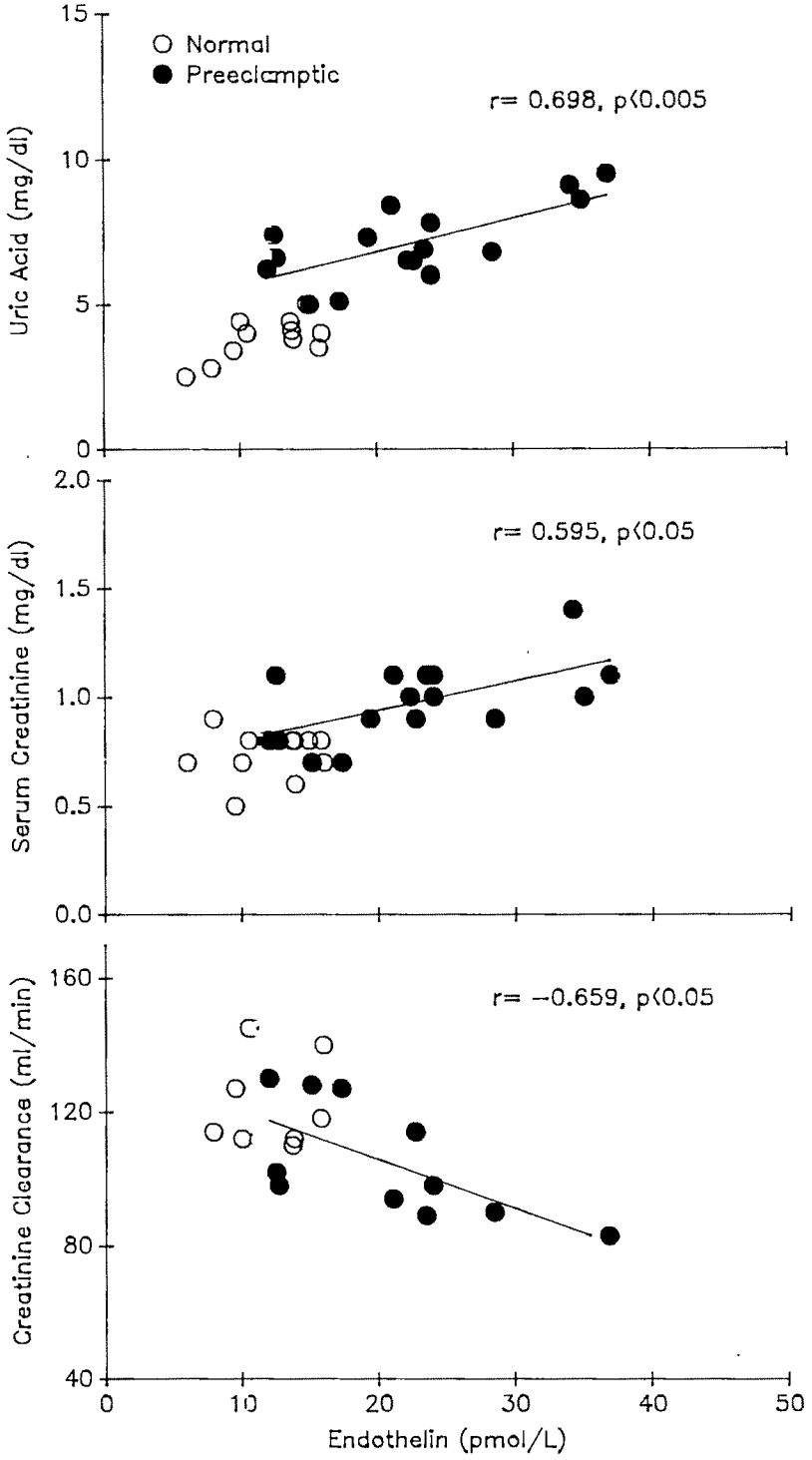


Fig. 1. Endothelin levels correlated significant with uric acid ($r = 0.698, p < 0.005$), serum creatinine ($r = 0.595, p < 0.05$), and creatirine clearance ($r = -0.659, p < 0.05$) in preeclamptic group *(closed circles)* but not in normal women *(open circles)*.

terial blood pressure was 76 ± 2 mm Hg. Endothelin levels averaged 17.7 ± 2.0 pmol/L, higher than those of the normal women of 27 to 38 weeks' gestation ($p < 0.05$) and lower than those of the preeclamptic group of 27 to 38 weeks' gestation, although this did not reach the level of statistical significance. Uric acid levels were 4.8 ± 0.4 mg/dl, higher than those of the earlier gestation normal women ($p < 0.05$) and lower than those of the women with preeclampsia ($p < 0.05$). Endothelin levels correlated positively with weeks of

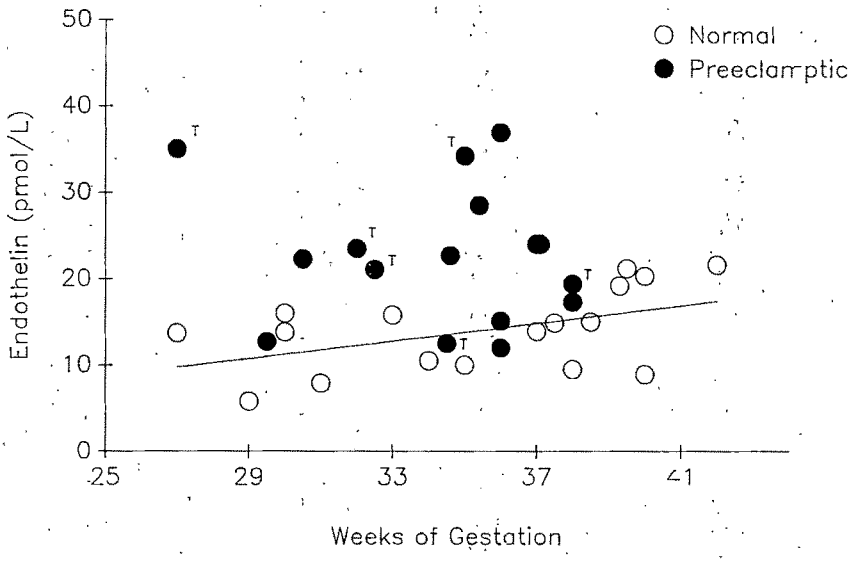


Fig. 2. Influence of weeks of gestation on endothelin levels. For any given gestational duration, endothelin levels were higher in preeclamptic group *(closed circles)* and rose significantly with length of gestation in normal pregnant women *(open circles)* ($r = 0.492$, $p < 0.05$). *T*, Twin gestation.

gestation over the third trimester in the 17 normal primigravid women ($r = 0.492$, $p < 0.05$). There was no correlation of endothelin levels with weeks of gestation in the preeclamptic group, although there were no women with preeclampsia beyond 38 weeks' gestation.

Comment

This study demonstrates elevated endothelin levels in women with preeclampsia compared with pregnant women of similar gestational duration without preeclampsia and with healthy, young, nonpregnant controls. Since the institution of this study there has been at least one other report of an increase in endothelin levels in women with preeclampsia.[13] However, the relevance of endothelin to the changes in renal function, urate resorption, and blood pressure seen in preeclampsia has not previously been examined. Endothelin levels in preeclamptic women had an especially close correlation to the serum uric acid level, serum creatinine, and creatinine clearance but did not correlate with mean arterial blood pressure or degree of proteinuria. Endothelin levels also seem to increase with gestational duration during the third trimester, with the major increase occurring close to the estimated delivery date.

For several reasons, the circulating level of endothelin is probably an imprecise guide to its physiologic role. We found a close correlation between endothelin and serum creatinine and creatinine clearance in preeclamptic women, suggesting but not proving a causal relationship. It seems possible that endothelin may be responsible in part for the reduction in glomerular filtration rate by increased renal vascular resistance. The kidney is appreciably more sensitive than other vascular regions to the vasoconstrictor effects of endothelin.[14] In animal experiments[15] and in the isolated kidney,[5] levels of endothelin (40 to 80 pmol/L) close to those found in women with preeclampsia are associated with renal vasoconstriction. In our laboratory infusion of low doses of endothelin into rats, to produce plasma levels of 15 to 30 pmol/L above basal levels, were capable of reducing renal perfusion approximately 25% (unpublished observations).

High-affinity binding sites for endothelin-1 have been demonstrated in both the glomerular and the placental circulations.[6-9, 16] Furthermore, sustained elevations in vascular resistance persist after endothelin infusion, long after circulating levels have returned to baseline, suggesting prolonged binding of endothelin to its receptors.[2-4] Therefore it is possible that even lower circulating plasma levels may have pathophysiologic significance. The observation of elevated endothelin levels and their correlation to measures of renal dysfunction, coupled with the presence of high-affinity binding sites for endothelin in the uteroplacental and glomerular circulation, suggest that endothelin may contribute to the declining renal function and diminished placental perfusion that are characteristic of preeclampsia.

A classic feature of the preeclamptic state is an elevated plasma level of uric acid, which is generally predictive of severity of disease[17, 18] and is closely correlated

with the glomerular endothelial hyperplasia characteristic of preeclampsia.[10] In the current study endothelin levels had a remarkably close correlation with plasma uric acid levels. Elevated uric acid levels in preeclampsia are thought to be the result of a decrease in uric acid clearance that is disproportionate to the fall in glomerular filtration rate.[11] The effect of endothelin on uric acid clearance in humans has not been measured; however, other vasoconstrictors, such as angiotensin II and norepinephrine,[19] have been shown to increase urate resorption, and endothelin might have a similar effect. Furthermore, endothelin has been recently shown to increase proximal tubule fluid reabsorption, the site of uric acid reabsorption.[20]

High levels of endothelin have been reported in umbilical vessels and fetal blood.[6-9] It has been postulated that endothelin may be important in the vasoconstriction of placental and umbilical vessels at the time of delivery, to prevent excessive blood loss.[6] If this were true, one might expect circulating endothelin levels to increase near the end of gestation. Indeed, we did find a positive correlation between weeks of gestation and endothelin levels in normal primigravid women. The presence of mildly elevated endothelin levels near term in normal pregnancy without evidence of vasoconstriction suggests that endothelin is unlikely to be the only variable contributing to renal vasoconstriction. There are clearly factors, such as enhanced platelet aggregability, diminished prostacyclin production, increased thromboxane levels, and other unknown variables, that may contribute to the difference in vasoconstrictive manifestations.

In the current study women with preeclampsia showed wide variability in ANP levels, a direct correlation between ANP levels and mean arterial blood pressure in the preeclamptic group, and no significant differences in levels between pregnant and nonpregnant women. Other studies of ANP levels in pregnancy report little difference between normal pregnant and nonpregnant controls, modestly higher levels in preeclampsia than in normal pregnancy, and positive correlations between ANP level and blood pressure.[21-25] Therefore, in spite of the well-known physiologic hypervolemia of pregnancy, ANP levels do not appear to be uniformly elevated.

In summary, circulating plasma endothelin levels are elevated in preeclampsia as compared with normal pregnancy and nonpregnancy. These levels are closely correlated with markers of disease severity and deteriorating renal function such as plasma urate, serum creatinine, and creatinine clearance. Endothelin levels also appear to rise late in gestation in normal pregnant women. Endothelin may be involved in the systemic vasoconstriction, reduction in renal and placental blood flow, and elevated plasma urate levels associated with preeclampsia.

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Latex anaphylaxis in an obstetrics and gynecology physician

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Latex anaphylaxis caused by surgical gloves developed in a physician with a history of urticaria to latex. Standard therapy with epinephine and fluids was effective. Latex allergy should be included in the differential diagnosis for anaphylaxis in a patient or a physician. (AM J OBSTET GYNECOL 1992;166:968-9.)

Key words: Anaphylaxis, latex, health care worker

Allergic reactions to latex are recognized with increasing frequency among health care workers.[1] We report a case of latex anaphylaxis developing in an obstetrician-gynecologist.

Case report

A 30-year-old resident physician in obstetrics and gynecology had a history of allergic reactions to latex-containing gloves. At age 16 contact dermatitis developed while she was wearing latex gloves as a nurse's aide. During medical school the allergic reaction progressed to local contact urticaria and mild systemic symptoms consisting of anxiety and dysphoria. The symptoms were controlled by the use of cotton and vinyl liners worn under the gloves. She had a history of eczema, allergic rash to ibuprofen, and allergic rhinitis. She had no other medical conditions.

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During her residency she was called to perform an emergency vaginal breech delivery. She put on latex gloves without the protective liners because of the urgency of the situation. During repair of the episiotomy, localized pruritus gradually developed on the hands, and she experienced dyspnea, tachypnea, extreme anxiety, dysphoria, and confusion. She removed the gloves after the repair was completed. Generalized erythema and swelling developed. Blood pressure was 70/30 mm Hg, pulse 110 beats/min, and respirations 30 breaths/min. (Normal values are blood pressure 110/60 mm Hg and pulse of 60 beats/min.) A capillary blood glucose level was 81 mg/dl. Anaphylactic shock was diagnosed. Standard treatment with vigorous intravenous fluid and two doses of epinephine 1:1000 concentration, 0.3 mg, was administered subcutaneously. The physician was transferred to the emergency room during the course of therapy. In addition, 50 mg diphenhydramine was given intramuscularly and 5 mg diazepam intravenous. The episode gradually resolved over a 3-hour period.

Skin testing confirmed a hypersensitivity reaction to latex. The physician now routinely uses nonlatex hypoallergenic gloves (Elastyren, Danpren, Denmark; Tactylon, Smartpractice, Phoenix). There has been no recurrence with this preventative measure.

Comment

The increasing use of latex gloves to protect health care workers is likely to be responsible for the increase in exposure and allergy to latex. Hypersensitivity to rubber products, including latex, has been recognized for more than a decade.[2] The allergic reaction may range from local contact dermatitis to anaphylaxis. The urticaria and anaphylaxis are immunoglobulin E mediated; the antibody may be directed either to latex itself or toward the chemicals used in the vulcanizing process.

The treatment of latex anaphylaxis does not differ from the treatment of anaphylaxis of other causes. Essential therapy consists of three measures:

1. Epinephrine. For airway or cutaneous reactions, use 0.3 to 0.5 ml of 1:1000 dilution given subcutaneously every 10 to 20 minutes as needed. Hypotension can be treated with 1 ml of 1:1000 dilution in 50 ml of crystalloid solution at a rate of 0.25 to 2.5 ml/min (0.5 to 5 μg/min).
2. Intravenous fluids. Hypotension may require 1 L of crystalloid solution every 20 to 30 minutes.
3. Oxygen. Give 40% to 100% to maintain an arterial partial pressure of oxygen of ≥60 mm Hg.

Recognition of an anaphylactic reaction is critical. Both the patient and health care worker are at risk for latex allergic reactions. Many commonly used products contain latex, including surgical gloves, catheters, enema devices, stoma bags, condoms, balloons, and rubber bands. For example, persons with spina bifida frequently are exposed to latex because of the need for long-term catheterization, and they are at increased risk for latex anaphylaxis. A history of allergic symptoms that develop when a toy balloon is inflated may help identify a latex allergy. Skin testing is a sensitive method to confirm latex hypersensitivity. The use of nonlatex surgical gloves or glove liners is a simple and effective method to prevent allergic episodes.

In summary, the prompt recognition and therapy of latex allergy are essential for the well-being of both patients and health care workers. Latex anaphylaxis should be included in the differential diagnosis of urticaria, bronchospasm, and hypotension.

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Does amniotic fluid analysis reflect acid-base balance in fetal blood?

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OBJECTIVE: The purpose of the study was to examine whether the acid-base balance in amniotic fluid reflects the acid-base state in fetal blood.
STUDY DESIGN: The pH, Pco_2, Po_2, bicarbonate, and base excess concentrations were measured in umbilical venous, umbilical arterial, and maternal venous blood and in amniotic fluid samples obtained at fetoscopy. This was performed before intraamniotic termination of 16 normal pregnancies (mean gestation 19.4 weeks), as well as in 14 red blood cell isoimmunized pregnancies (mean gestation 28.4 weeks) undergoing fetoscopy for the diagnosis and treatment of fetal anemia.
RESULTS: In normal pregnancies, amniotic fluid pH and bicarbonate were significantly lower than umbilical venous and umbilical arterial blood concentrations, and this was due to the high concentration of base deficit in amniotic fluid. Whereas amniotic fluid Pco_2 did correlate significantly with fetal umbilical venous Pco_2 values, there was no correlation between other acid-base characteristics.
CONCLUSION: Simultaneous sampling of human fetal umbilical blood and amniotic fluid under the acute conditions of this study did not show significant correlations in acid-base values. (AM J OBSTET GYNECOL 1992;166:970-3.)

Key words: Acid-base balance, amniotic fluid, fetal blood, fetoscopy, red blood cell isoimmunization

Fetal blood sampling was originally introduced for the prenatal diagnosis of inherited disorders, but most recently it has been used for assessing fetal well-being.[1, 2] Measurement of blood gases and acid-base status by umbilical cord sampling is the only direct method of diagnosing asphyxia in utero. However, ultrasonographically guided fetal blood sampling requires considerable expertise, is not widely available and can also be difficult in the presence of oligohydramnios. In addition, with cordocentesis either the umbilical vein or artery can be sampled, so physiologic observations regarding placental transfer of oxygen and nutrients to the fetus can be limited. The aim of the current study was to assess whether examination of amniotic fluid, which is more easily obtained than fetal blood, represents acid-base balance and oxygenation of the fetus. To achieve this, amniotic fluid and paired umbilical arterial and umbilical venous blood samples obtained at fetoscopy from midtrimester pregnancies were examined.

Patients and methods

Fetoscopy and fetal blood sampling were preformed in 16 pregnancies before the instillation of prostaglandins for elective abortion at 16 to 24 weeks' gestation. The patients (mean gestation 19.7 weeks) were undergoing termination for social reasons under clause two of the Abortion Act 1967. Fetoscopy was also performed on 14 red blood cell–isoimmunized pregnancies at 18 to 36 weeks' gestation (mean gestation 28.4 weeks) for the diagnosis and treatment of fetal anemia. None of the fetuses was hydropic. Informed consent was obtained from all patients before fetoscopy. Ethics committee support for the principle of fetal blood sampling at the time of termination had previously been obtained. The fetuses were subsequently confirmed at postabortion autopsy to be structurally and chromosomally normal.

Fetoscopy was performed as previously described.[3] Amniotic fluid (10 ml) and fetal blood (1 to 2 ml) from the umbilical vein or artery were obtained under direct vision. In 10 termination cases samples from both the umbilical artery and vein were obtained. Maternal venous blood (10 ml) was also collected from an antecubital vein distal to a tourniquet at the time of fetoscopy.

All samples were collected into preheparinized plastic syringes, and with the Radiometer ABL automatic analyzer (Radiometer, Copenhagen), analyses were performed within 30 minutes of collection. The pH, Pco_2, and Po_2 were measured, and bicarbonate and base deficit were extrapolated. All cord samples were confirmed to be fetal in origin by Kleihauer-Betke testing.

Results were analyzed with the Statistical Package for Personal Computers (P. Royston, London). Regression

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Table I. Means and standard deviations of pH, P_{CO_2}, P_{O_2}, base excess, and bicarbonate concentration in amniotic fluid, umbilical venous and arterial blood, and maternal venous blood in normal pregnancies ($n = 16$) and those complicated by red blood cell isoimmunization ($n = 14$)

	Amniotic fluid	*Umbilical venous blood*	*Umbilical arterial blood*	*Maternal venous blood*
Normal pregnancies				
No.	16	15	11	16
pH	7.18 ± 0.05	7.37 ± 0.03	7.31 ± 0.02	7.37 ± 0.04
P_{CO_2}	43.4 ± 2.7	37 ± 3.6	46 ± 6.1	38.4 ± 5.8
P_{O_2}	86.3 ± 20.7	44.3 ± 7.3	21.7 ± 5.2	42.3 ± 18
Bicarbonate	15.9 ± 1.6	21.5 ± 1.9	23 ± 3.0	21.8 ± 1.9
Base excess	−10.3 ± 1.9	−3.1 ± 1.3	−3.1 ± 2.6	−2.8 ± 1.4
Isoimmunized pregnancies				
No.	14	7	10	12
pH	7.14 ± 0.05*	7.34 ± 0.03*	7.31 ± 0.03	7.37 ± 0.03
P_{CO_2}	47.5 ± 4.6*	37.7 ± 7.4	47.6 ± 5.5	37.5 ± 3.6
P_{O_2}	90.6 ± 14.5	43.0 ± 8.7	22.5 ± 4.7	44.1 ± 10.4
Bicarbonate	15.4 ± 1.4	20.4 ± 1.6	23.4 ± 2.3	21.6 ± 1.7
Base excess	−11.1 ± 1.7	−4.2 ± 1.7	−2.7 ± 1.8	−2.7 ± 1.9

*Significantly different ($p < 0.05$) from normal pregnancies.

analysis was used to test for any correlations between maternal, fetal, and amniotic fluid samples. The paired Student t test was used to test for any differences between amniotic fluid and blood values. Normal and red blood cell–isoimmunized values were compared with the Mann-Whitney test.

Results

The pH, P_{CO_2}, P_{O_2}, base deficit, and bicarbonate concentrations in amniotic fluid, umbilical arterial and venous, and maternal venous blood samples in normal and red blood cell–isoimmunized human pregnancies are summarized in Table I.

In normal pregnancies the amniotic fluid pH was significantly lower than both the umbilical venous pH ($t = 17.4$, $p < 0.0001$) and the umbilical arterial pH ($t = 10.3$, $p < 0.0001$), and the umbilical arterial pH was significantly lower than the umbilical venous pH ($t = 9.75$, $p < 0.0001$). In addition, the amniotic fluid base deficit was significantly higher than both the umbilical venous base deficit ($t = 12$, $p < 0.0001$) and the umbilical arterial base deficit ($t = 7.81$, $p < 0.0001$). The amniotic fluid bicarbonate concentration was significantly lower than the umbilical venous bicarbonate ($t = 11.87$, $p < 0.0001$), umbilical arterial bicarbonate ($t = 7.91$, $p < 0.0001$), and maternal venous bicarbonate ($t = 14.7$, $p < 0.0001$).

Carbon dioxide tension was significantly higher in amniotic fluid than in umbilical venous blood ($t = 9.18$, $p < 0.001$) but not significantly different from umbilical arterial P_{CO_2}. However, the umbilical arterial P_{CO_2} was significantly higher than both the umbilical venous P_{CO_2} ($t = 6.14$, $p < 0.001$) and the maternal venous P_{CO_2} ($t = 2.39$, $p < 0.05$). The mean arteriovenous P_{CO_2} difference was 9.9 mm Hg in paired umbilical samples. The amniotic fluid P_{O_2} was significantly higher than both umbilical venous P_{O_2} ($t = 6.72$, $p < 0.0001$) and umbilical arterial P_{O_2} ($t = 9.83$, $p < 0.0001$). The umbilical venous P_{O_2} was significantly higher than the umbilical arterial P_{O_2} ($t = 13.2$, $p < 0.0001$), and the mean arteriovenous P_{O_2} difference in paired samples was 23.4 mm Hg.

The correlations between amniotic fluid, umbilical venous and arterial, and maternal venous pH, P_{CO_2}, P_{O_2}, base deficit, and bicarbonate in normal and isoimmunized pregnancies at midgestation are shown in Table II. There were significant correlations between amniotic fluid and umbilical venous P_{CO_2} ($r = 0.64$, $p < 0.001$), amniotic fluid and maternal venous bicarbonate ($r = 0.65$, $p < 0.001$), and amniotic fluid and umbilical arterial bicarbonate ($r = 0.45$, $p < 0.05$).

The isoimmunized pregnancies (Table I) had significantly lower amniotic fluid pH ($p < 0.01$) and lower umbilical venous pH ($p < 0.05$) but significantly higher amniotic fluid P_{CO_2} ($p < 0.01$).

Comment

The gas and pH measurements in fetal blood samples obtained at fetoscopy from normal midtrimester human fetuses have been reported previously.[4] The results are in general agreement with those of Soothill et al.,[5] who also reported on fetoscopically obtained samples in midpregnancy. However, in our study, the umbilical venous pH was higher, the umbilical arterial pH was lower, and both umbilical venous and umbilical arterial P_{O_2} were lower. The same authors found that samples obtained fetoscopically were more acidotic and hypercapnic than those obtained at cordocentesis.[6] This was attributed to maternal fasting and sedation, although the maternal arterial blood gases measured at the time of fetoscopy were not abnormal.

Ultrasonographically guided needling of the umbilical cord is now the method of choice for fetal blood sampling, but the advantage of fetoscopy is that paired

Table II. Correlations between amniotic fluid, umbilical venous and arterial blood, and maternal venous blood pH, P_{CO_2}, P_{O_2}, base excess, and bicarbonate in normal and isoimmunized pregnancies at midgestation

	pH	P_{CO_2}	P_{O_2}	*Base excess*	*Bicarbonate*	*n*
Normal pregnancies						
Amniotic fluid versus umbilical venous blood	−0.07	0.675*	0.04	0.04	0.50	15
Amniotic fluid versus umbilical arterial blood	−0.17	0.10	0.29	0.22	0.50	11
Amniotic fluid versus maternal venous blood	−0.16	−0.50†	−0.04	−0.11	0.59†	16
Maternal venous versus umbilical venous blood	0.57†	0.54†	−0.01	0.33	0.65*	15
Maternal venous versus umbilical arterial blood	0.46	−0.01	0.04	0.13	0.41	11
Umbilical venous versus umbilical arterial blood	0.77*	−0.03	0.65*	0.07	0.23	10
Normal and isoimmunized pregnancies						
Amniotic fluid versus umbilical venous blood	0.40	0.64‡	0.08	0.02	0.40	22
Amniotic fluid versus umbilical arterial blood	0.04	0.35	0.13	0.32	0.45†	21
Amniotic fluid versus maternal venous blood	−0.09	0.18	−0.11	0.39†	0.65‡	28
Maternal venous versus umbilical venous blood	0.43†	0.30	0.07	0.34	0.63*	22
Maternal venous versus umbilical arterial blood	0.45†	0.08	0.32	0.41	0.59*	21
Umbilical venous versus umbilical arterial blood	0.50	0.06	0.64*	−0.11	0.10	17

*$p < 0.01$.
†$p < 0.05$.
‡$p < 0.001$.

umbilical arterial and venous samples may aid our understanding of fetal physiologic characteristics. Thus the umbilical arteriovenous oxygen difference in paired samples in the midtrimester human fetus is 23 mm Hg. Assuming that the mean umbilical venous blood flow at 20 weeks' gestation is 0.15 L/kg/min,[7] then fetal oxygen consumption in midpregnancy can be estimated to be 0.59 mmol/kg/min. This is higher than that measured for the horse (0.31 mmol/kg/min), rhesus monkey (0.31 mmol/kg/min), or guinea pig (0.40 mmol/kg/min).[8]

There were significant correlations between maternal venous pH and umbilical venous or umbilical arterial pH. Similarly, the maternal venous bicarbonate concentration was correlated with both the umbilical blood and amniotic fluid bicarbonate concentrations. Therefore in interpreting the fetal acid-base status one must first confirm a normal acid-base balance in the mother.

The mean pH of amniotic fluid in midtrimester (7.18) is similar to that reported previously,[9,10] although higher than that reported by Rooth et al. (7.12).[11] Amniotic fluid was acidotic and had a high base deficit. This is because of the high concentration of organic acids, especially lactic acid, and also because of its low buffering capacity compared with that of fetal blood.[12] The implication of low amniotic fluid pH is that contamination of fetal blood samples with amniotic fluid, especially in cases of oligohydramnios and intrauterine growth retardation, when cordocentesis might be difficult, could give misleadingly low blood pH values, although this has not been tested in vitro. Not only is amniotic fluid acidotic compared with fetal blood but, in addition, there was no significant correlation between amniotic fluid and umbilical blood pH.

The amniotic fluid P_{CO_2} values are similar to those reported by other investigators.[10] The partial pressure of carbon dioxide in amniotic fluid was not different from that in the umbilical artery. In addition, there was a significant correlation between amniotic fluid and umbilical venous P_{CO_2} and amniotic fluid and maternal venous P_{CO_2}. This is not surprising because carbon dioxide has the ability to diffuse rapidly between fluid compartments.

The amniotic fluid P_{O_2} (mean 86.3 mm) is much higher than that reported in other studies, but there is great variability in the values reported by all the authors who studied this subject (means 2.8 to 99 mm Hg in different studies).[13] However, there are well-known difficulties in measuring P_{O_2} in amniotic fluid, and results are reliable only if the samples are drawn into glass

syringes and stored near the freezing point.[9] In addition, the oxygen content of amniotic fluid is insignificant compared with that of fetal blood because oxygen in amniotic fluid is soluble and not bound to a carrier protein such as hemoglobin. For these reasons, P_{O_2} of amniotic fluid would not be helpful in assessing fetal blood oxygenation.

The isoimmunized pregnancies were more advanced in gestation (mean gestation 28 weeks), so caution must be used when the results are compared with those of the normal pregnancies (mean gestation 19.4 weeks). The amniotic fluid pH was lower and the P_{CO_2} was higher in isoimmunized than in normal pregnancies. However, there was also a comparable decrease in umbilical venous pH, and, because umbilical venous pH and arterial pH decrease and P_{CO_2} increases with gestation in normal fetuses,[14] the pH and P_{CO_2} differences in the two groups might be explained by the effects of gestation. Indeed, 6 weeks before term the pH of amniotic fluid from normal pregnancies is 7.10 and the mean P_{CO_2} 53 mm Hg,[15] indicating that there is a progressive increase in amniotic fluid P_{CO_2} and a concomitant decrease in amniotic fluid pH with gestation. Alternatively, fetal anemia, if severe, could lead to fetal acidosis as a result of lactic acid accumulation.[16]

Simultaneous sampling of normal human fetal umbilical blood and amniotic fluid under the acute conditions of this study did not show significant correlations in acid-base values. It is possible that the time course for change in the amniotic fluid might be so slow that changes in blood values are not immediately observed in the amniotic fluid pool. Whether amniotic fluid acid-base balance would be helpful in the assessment of the chronically acidotic fetus remains to be seen.

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Appendix

Calculation of fetal oxygen consumption

Formula 1:

$$\text{Fractional saturation} = \frac{K \times {Po_2}^{2.8}}{1 + K \times {Po_2}^{2.8}}$$

where

$$K = \frac{1}{{P_{50}}^{2.8}}$$

and

$$P_{50} = 21 \times 10^{[-0.48 \times (7.4 - pH)]}$$

Formula 2:

$$\text{Oxygen content} = \text{Hemoglobin (gm/dl)} \times \text{Fractional saturation} \times 0.5982$$

The umbilical arteriovenous oxygen difference was found to be 23.4 mm Hg. Assuming that the hemoglobin concentration at 20 weeks' gestation is 11.7 gm/dl,[17] then the amount of umbilical oxygen uptake can be calculated to be 3.94 mmol/L. Assuming that the umbilical venous blood flow is 0.15 L/kg/min, then the fetal oxygen consumption at 20 weeks' gestation can be estimated to be $3.94 \times 0.15 = 0.59$ mmol/kg/min.

The clinical significance of thrombocytosis in women presenting with a pelvic mass

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OBJECTIVES: We attempted to determine the clinical usefulness of a preoperative platelet count for predicting malignancy in patients presenting with a pelvic mass. Additionally, in patients in whom a preoperative serum CA 125 level was also determined, the clinical usefulness was compared.
STUDY DESIGN: A retrospective review of charts of 241 patients presenting with a pelvic mass was carried out. The patients were then divided by pathologic diagnosis into group 1, benign disease (n = 102), and group 2, invasive (n = 121) or borderline (n = 18) malignancies. Thrombocytosis (platelet count >400,000/μl) was present in 16% (n = 16) of patients in group 1 and in 56% (n = 78) of patients in group 2 ($p < 0.0001$). Of the 241 patients, 130 also had preoperative serum CA 125 levels measured. Elevated values of serum CA 125 (>35 U/ml) occurred in 14% (n = 8) of group 1 and in 81% (n = 60) of group 2 ($p < 0.0001$). The χ^2 test was used to analyze results of data.
RESULTS: Statistically significant differences in preoperative platelet count and serum CA 125 values were found between group 1 and group 2 patients. The specificity and positive predictive value for thrombocytosis (84% and 83%) compared well with those for serum CA 125 (86% and 88%). The combined use of the tests resulted in specificity of 96% and positive predictive value of 95%.
CONCLUSIONS: Preoperative platelet count is a test that is rapidly available and easily obtained. The test results may be of value in planning the original management of women presenting with a pelvic mass. (Am J Obstet Gynecol 1992;166:974-7.)

Key words: Thrombocytosis, pelvic mass, CA 125

In evaluating and managing a woman with a pelvic mass, serious consideration must be given to the risk of ultimately finding a malignancy. Clinical presentation, patient age, and physical findings may suggest the etiology but can be misleading. Similarly, radiologic evaluation is most accurate with patients having advanced cancer with extraperitoneal or massive intraperitoneal disease, which clinical examination often accurately detects. The patients with early disease or minimal intraperitoneal disease pose the greatest challenge.

The choice of incision, extent of surgery, risk of intraoperative or postoperative complication, and potential need for referral to a tertiary care center are all related to the diagnosis of cancer. Attempts at selecting patients likely to have cancer by relying on serum CA 125 levels are suboptimal because of a significant false-negative rate with early disease and a false-positive elevation related to endometriosis, pelvic inflammatory disease, and other diseases.[1, 2]

One of the authors (W.M.) observed the frequent finding of thrombocytosis in patients presenting with a pelvic mass who were subsequently found to have cancer at exploration. A review of our 10 years' experience suggests that thrombocytosis, if associated with an elevated serum CA 125 level, may improve the preoperative diagnosis of ovarian cancer and assist in management decisions and patient counseling.

Table I. Pathologic diagnoses, group 1

Ovarian mass		70
Functional cyst	22	
Epithelial tumors	24	
Serous cystadenoma or adenofibroma	14	
Mucinous cystadenoma or adenofibroma	9	
Brenner tumor	1	
Endometrioma	10	
Mature teratoma	6	
Thecoma or fibroma	4	
Tuboovarian abscess	4	
Tubal mass		5
Hydrosalpinx or pyosalpinx	4	
Paratubal cyst	1	
Uterine mass		22
Leiomyoma	22	
Peritoneal pathologic conditions		4
Pseudomyxoma peritonei associated with benign mucinous lesion	2	
Endometriosis of abdominal wall scar	1	
Mesothelial cyst	1	
Bowel mass		1
Diverticular abscess	1	
TOTAL		102

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Table II. Pathologic diagnoses, group 2

Ovarian mass			132
Epithelial ovarian malignancies		107	
Serous	66		
Mucinous	12		
Clear cell	13		
Endometrioid	11		
Papillary mixed	5		
Epithelial ovarian tumors of low malignant potential		18	
Serous	9		
Mucinous	7		
Endometrioid	1		
Papillary mixed	1		
Germ cell malignancies		4	
Dysgerminoma	2		
Immature teratoma	1		
Endodermal tumor	1		
Sex cord stromal tumor		3	
Granulosa cell tumor	1		
Sertoli-Leydig cell tumor	2		
Peritoneal pathologic conditions			2
Peritoneal primary	1		
Malignant mesothelioma	1		
Other (sarcoma, lymphoma, unknown primary)			5
TOTAL			139

Material and methods

Records of 288 patients, seen by the Gynecologic Oncology Service at the State University of New York at Stony Brook, from May 1980 to April 1990 with a pelvic mass were reviewed. Forty-seven patients were excluded from our study for the following reasons: (1) lack of preoperative platelet count and (2) the presence of underlying conditions associated with thrombocytosis (i.e., asplenism, collagen vascular disease, postoperative and postpartum states). No patients had a history of administration of drugs known to cause thrombocytosis. We defined thrombocytosis as a platelet count >400,000/μl, in agreement with other studies.[3-5] Serum CA 125 values >35U/ml were considered abnormal.[1]

All patients underwent exploratory laparotomy, and the surgical specimens were reviewed by a gynecologic oncologist and a gynecologic pathologist. Final pathologic results were used to assign patients to one of two groups: group 1, benign disease (N = 102), and group 2, invasive (n = 121) and borderline ovarian (n = 18) malignancies. The clinical usefulness of elevated platelet count and serum CA 125 levels was assessed by calculating sensitivity, specificity, and positive and negative predictive values, and the null hypothesis was tested with χ^2 analysis.

Results

The pathologic diagnoses of the masses in patients are summarized in Table I and II. The majority of benign masses were adnexal in origin, whereas most patients in group 2 had epithelial ovarian adenocarcinomas. Thrombocytosis was noted in 16 (16%) of the patients in group 1 (Table III) and in 78 (56%) of the patients in group 2 (Table IV). The difference in incidence of thrombocytosis was found to be highly statistically significant ($p < 0.0001$). Table IV also lists the incidence of thrombocytosis in patients with epithelial ovarian malignancy (n = 126) by stage (International Federation of Gynecology and Obstetrics, 1988).

Table III. Diagnoses in patients with abnormal values, group 1

	Thrombocytosis	*Elevated CA 125**
Benign adnexal masses		
Tuboovarian abscess	4 (100%)	1 (50%)
Functional cysts (one torted)	3 (13.6%)	2 (12.5%)
Serous cystadenoma or adenofibroma (one torted)	3 (21.4%)	2 (20%)
Mucinous cystadenoma or adenofibroma	1 (11.1%)	1 (14.3%)
Endometrioma	1 (10%)	2 (50%)
Uterine masses		
Leiomyoma (one atypical and one torted)	4 (18.1%)	0 (0%)
TOTAL	16	8

*Serum CA 125 values were not obtained from all patients.

Table IV. Diagnoses in patients with abnormal values, group 2

	Thrombocytosis	*Elevated CA 125**
Epithelial ovarian malignancies		
Stage I	11 (44%)	8 (53%)
Stage II	2 (50%)	2 (67%)
Stage III	39 (61%)	31 (97%)
Stage IV	22 (69%)	15 (94%)
Immature teratoma	1 (100%)	1 (100%)
Sertoli-Leydig cell	0 (0%)	1 (50%)
Mesothelioma	1 (100%)	1 (100%)
Primary peritoneal carcinoma	1 (100%)	1 (100%)
Ovarian sarcoma	1 (100%)	—
TOTAL	78	60

*CA 125 values were not obtained from all patients.

Preoperative serum CA 125 values were not available in all patients. However, elevated levels were noted in 8 of 56 (14%) patients in group 1 and in 60 of 74 (81%) in group 2 (Tables III through V); the difference was again highly significant ($p < 0.0001$).

Table VI lists the sensitivity, specificity, and positive and negative predictive values of thrombocytosis, serum CA 125 levels, and both tests in patients presenting with pelvic masses. Additionally, patients in groups 1 and 2 were subdivided by age (Table VII). When compared with younger patients, patients >50 years old with thrombocytosis had a higher positive predictive value and sensitivity and specificity, but the negative predictive value for the test was decreased. Throm-

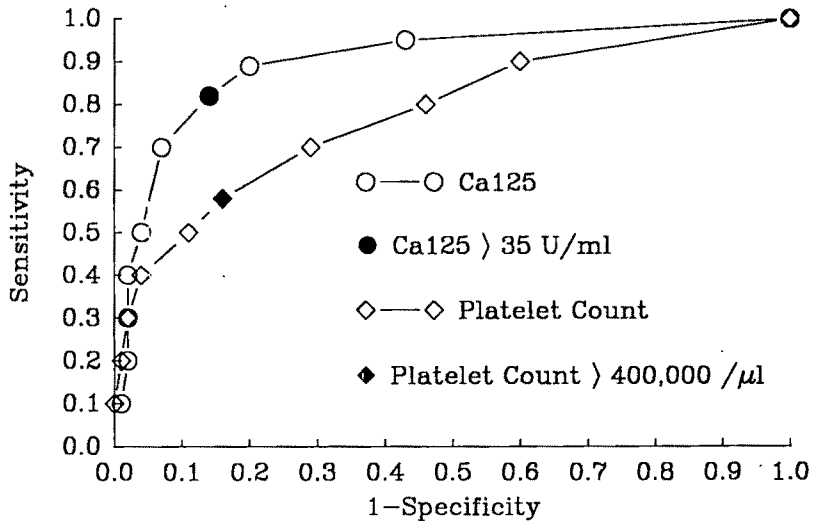


Fig. 1. Receiver-operator characteristic curve for platelet count and CA 125.

Table V. Incidence of thrombocytosis, elevated serum CA 125 levels and both values in groups 1 and 2

Population	*Thrombocytosis*	*CA 125*	*Both*
Group 1	16 (16%) (n = 102)	8 (14%) (n = 56)	2 (4%) (n = 56)
Group 2	78 (56%) (n = 139)	60 (81%) (n = 74)	37 (50%) (n = 74)

Table VI. Statistical parameters for thrombocytosis, CA 125 levels, and the combined tests

	Thrombocytosis (%)	*CA 125 (%)*	*Both (%)*
Sensitivity	56	81	50
Specificity	84	86	96
Positive predictive value	83	88	95
Negative predictive value	59	77	39

bocytosis was not seen after operation or during chemotherapy. Patients with recurrent disease did not have thrombocytosis. Fig. 1 depicts the receiver-operator characteristic curves for platelet counts and serum CA 125 values. Although serum CA 125 level is a superior test, platelet count >400,000/μl appears to represent a clinically useful marker.

Comment

Ginsburg[4] defined thrombocytosis as a moderately short-lived and symptomless elevation of platelet count occurring in association with certain underlying diseases. A specific stimulus for thrombocytosis has not been identified. Proposed theories include tumor-related humoral factors with thrombopoietin-like activity[6] and a tumor-stimulated state of overcompensated disseminated intravascular coagulopathy.[7-9] Thrombocytosis has been shown to be associated with underlying malignancy in a number of studies.[6-11] When those with myeloproliferative disorders were excluded, 36% of patients with thrombocytosis were diagnosed as having a malignant neoplasm. Patients with ovarian cancer accounted for 20% of this population.[10] Other conditions (i.e., anemia, inflammatory bowel disease, trauma, etc.) can stimulate platelet production,[7] resulting in a lack of suitability of the test for screening purposes.

Table VII. Statistical parameters for thrombocytosis, CA 125 levels, and the combined tests by age

	Age (yr)	*Thrombocytosis (%)*	*CA 125 (%)*	*Both (%)*
Sensitivity	<50	50	74	44
	≥50	60	85	53
Specificity	<50	83	83	97
	≥50	87	88	96
Positive predictive value	<50	68	80	92
	≥50	92	93	96
Negative predictive value	<50	70	78	66
	≥50	47	77	53

The majority of patients with ovarian carcinoma are first seen with advanced disease.[12] Currently, there are many tumor markers that have been evaluated in patients with ovarian carcinoma.[13-18] Monitoring serum CA 125 levels has become standard in these patients, but a sensitive screening tool to detect or predict the presence of ovarian carcinoma is not yet available. Recently, the use of vaginal ultrasonography with serum CA 125 values has shown some promise.[19-21] We have

demonstrated that thrombocytosis found in patients presenting with a pelvic mass is a highly significant finding, with up to 50% of patients with stage I and stage II ovarian malignancies having thrombocytosis. Although thrombocytosis was found in 16% (n = 16) of patients in group 1, seven of these patients had pathologic conditions that could result in stimulation of platelet production (four with inflammatory process, two with adnexal torsion, and one with infarcted leiomyoma), and an additional five patients were found to have a benign neoplasm (four with cystadenomas and one endometrioma).

The specificity and positive predictive values for thrombocytosis compared quite well with those of preoperative serum CA 125 values obtained in patients in our study, but the sensitivity of the test is poor. In addition, a comparison of the statistical parameters of thrombocytosis with previously published values for serum CA 125 in predicting the presence of malignancy in patients with a pelvic mass was favorable.[1, 12, 22-25] In patients >50 years old, when the incidence of most gynecologic malignancies increases, the positive predictive value of thrombocytosis was obviously improved. Finally, in the subgroup of patients where a preoperative CA 125 value was also available, the use of combined tests resulted in a very high specificity (96%) and positive predictive value (95%).

Gynecologists are frequently presented with the difficulty of counseling a patient with a pelvic mass. Preoperatively, they must prepare the patient for the possibility of a malignancy and assess whether such a patient would benefit by referral to a specialist. Platelet count is easily performed and the result is rapidly available. Frequently, the test is obtained routinely as part of a complete blood cell count, but the results may go unnoticed or are not found to be clinically important. The combined use of platelet count and serum CA 125 values results in a very high specificity and positive predictive value.

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Unexplained elevated maternal serum α-fetoprotein is not predictive of adverse perinatal outcome in an indigent urban population

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OBJECTIVE: The null hypothesis of this study is that in an urban, indigent obstetric population at high risk for adverse perinatal outcome, unexplained elevations of maternal serum α-fetoprotein are not an additional predictor of adverse perinatal outcome.

STUDY DESIGN: Perinatal outcomes of 72 patients from a clinic for indigent patients with unexplained elevated maternal serum α-fetoprotein levels were compared with those of matched controls from the same population with normal maternal serum α-fetoprotein levels. Subjects and controls were matched for age, race, parity, and presence or absence of Hollister risk factors. The frequency of adverse perinatal outcome in the two groups was subjected to matched-pair χ^2 analysis.

RESULTS: Adverse perinatal outcome occurred in 38.9% (28 of 72) of subjects with unexplained elevated maternal serum α-fetoprotein levels ≥2.5 multiples of the median, compared with 31.9% (23 of 72) of controls with normal maternal serum α-fetoprotein levels ($p = 0.5$). No statistically significant difference in adverse perinatal outcomes was found.

CONCLUSIONS: Elevated maternal serum α-fetoprotein levels offer little if any additional predictive value for adverse perinatal outcome in populations already at high risk for such outcomes on the basis of obstetric or socioeconomic criteria. (AM J OBSTET GYNECOL 1992;166:978-82.)

Key words: Maternal serum α-fetoprotein, adverse perinatal outcome

Several studies have shown an association between adverse perinatal outcome and unexplained elevated levels of maternal serum α-fetoprotein (AFP).[1-5] Such adverse perinatal outcomes included fetal loss, preterm labor, preterm rupture of membranes, pregnancy-induced hypertension, stillbirth, intrauterine growth retardation (IUGR), and abruptio placentae.[1-5] Investigators logically have recommended that patients with unexplained elevated maternal serum AFP should be considered high risk for adverse perinatal outcome and should have very close, but as yet undefined, prenatal follow-up.[4-7]

These studies, however, were all conducted in obstetric populations that were predominantly low risk. For example, the study population of Milunsky et al.[6] was mostly white (94%) and received prenatal care from private obstetricians. The study populations of Burton[4] in North Carolina and Robinson et al.[5] in California were derived from regional neural tube defect screening programs that included private practice patients. Studies of Wald et al.,[1] Brock et al.,[2] and Smith[3] were from Great Britain, with demographic information not offered. In addition, matched-pair analysis was not performed in any of these studies.

The purpose of this study was to determine whether unexplained elevated maternal serum AFP would show additional predictive value in patients already identified to be high risk for adverse perinatal outcome on the basis of other obstetric or socioeconomic criteria.

Material and methods

Subjects and controls, to be defined below, received obstetric care between Jan. 1, 1987, and Dec. 31, 1989, at the Regional Medical Center, Memphis, Tennessee. In 1990 this population was 79.6% black, 89% indigent (78% on Medicaid, 11% uninsured), and 61% unwed, with an estimated 10% incidence of maternal substance abuse. All patients were offered maternal serum AFP screening between 15 and 20 weeks.

Maternal serum AFP assays were performed with the Abbott enzyme immunoassay. An elevated maternal serum AFP level was defined as ≥2.5 multiples of the

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Table I. Unequivocal risk factors for adverse perinatal outcome*

Cardiac disease (class I to IV)	Smoking (>1 pack per day)
Tuberculosis, active	Prior fetal or neonatal death
Chronic pulmonary disease	Diabetes mellitus
Thrombophlebitis	Hypertension
Endocrinopathy	Chronic renal disease
Epilepsy (on medication)	Congenital-chromosomal anomalies
Two abortions (spontaneous)	Hemoglobinopathies
Previous preterm or small-for-gestational-age infants	Isoimmunization (Rh)
Isoimmunization (ABO, etc.)	Alcohol or drug abuse
Incompetent cervix	Habitual abortions
Previous preeclampsia	

*Adapted from Preexisting Risk Guide, Hollister Inc., Libertyville, Ill.

Table II. Risk factors that do not unequivocally place a patient at risk for adverse perinatal outcome*

Age <15 or >35 yr	<8th Grade education
≥7 Deliveries	Infants ≥4000 gm
Hemorrhage during previous pregnancy	Surgically scarred uterus
Pregnancy without familial support	Second pregnancy in 12 mo
Age ≥40 yr	Prior neurologically damaged infant
Significant social problems	Infertility

*Adapted from Preexisting Risk Guide, Hollister Inc., Libertyville, Ill.

median, adjusted for weight, race, and diabetes mellitus. If maternal serum AFP was 2.5 to 2.9 multiples of the median and the patient's estimated gestational age was ≤18 weeks, our policy was to repeat the assay 1 week later. If the repeat value was <2.5 multiples of the median, maternal serum AFP was considered normal and the patient was not included in the study. If a repeat sample was ≥2.5 multiples of the median, if the initial value was ≥3.0 multiples of the median, or if gestational age was ≥18 weeks, high-resolution ultrasonographic examination was performed. The 18-week cutoff was chosen to ensure that patients potentially carrying an anomalous fetus later in the second trimester would be evaluated in time that all pregnancy options could be offered, including termination. If gestational age that was based on ultrasonographic measurements differed from gestational age that was based on onset of last menses by >14 days, maternal serum AFP was reinterpreted with ultrasonographic dating; values reinterpreted as being within the normal range were considered explained. Amniocentesis was offered to patients having no ultrasonographic explanation for elevated maternal serum AFP levels. Patients who declined amniocentesis were included in our analysis; patients who declined ultrasonography or were found to have multiple gestations were excluded.

An elevated maternal serum AFP value was considered unexplained if, after correcting for erroneous gestational age, multiple gestation, and fetal death, no abnormality in fetal anatomy was detected on ultrasonographic examination. Patients who had oligohydramnios (<2 cm pocket of amniotic fluid on ultrasonography) were excluded because of the known association between oligohydramnios, unexplained elevated maternal serum AFP, and poor outcome.[8]

Standard Hollister forms (Hollister Inc., Libertyville, Ill.) were completed by perinatal nurses or house staff at the initial visit on all patients. This form permits information to be recorded concerning the patient's past medical history, family history, previous pregnancy outcomes, and presence or absence of 33 factors that help identify pregnancies at risk for adverse outcome. In our opinion 21 of the 33 risk factors unequivocally place a patient at increased risk for adverse outcome (Table I); however, the other 12 factors are social factors or other factors that do not, in our opinion, unequivocally place a patient at increased risk (Table II). Subjects of this study were thus subgrouped according to presence or absence of any of the 21 unequivocal medical risk factors (Table I).

According to a table of random numbers, controls were selected from patients who had singleton pregnancies and normal maternal serum AFP levels (<2.5 multiples of the median and with a Down syndrome risk less than that of a 35-year-old woman at midtrimester, i.e., 1 in 277). Subjects were matched for the presence or absence of any of 21 medical risk factors (i.e., risk factors present or not present), race (white vs black), age (5-year intervals), and parity (0 vs ≥1).

Pregnancy outcome data were gathered from hospital records by a single physician who was unaware of maternal serum AFP status at the time of review. Outcome variables specifically assessed were congenital malformations, spontaneous pregnancy loss <20 weeks, preterm labor (<37 weeks), preterm rupture of membranes (<37 weeks), IUGR, stillbirths, pregnancy-induced hypertension, and abruptio placentae. Statistical analysis was done by McNemar's test (matched-pair χ^2 test),[9] a test that minimizes the effects of confounding variables.

Results

Among 6208 patients undergoing maternal serum AFP screening during the study period, 88 (1.4%) had unexplained elevated values; three of the 88 were excluded because multiple gestation, four because of oligohydramnios, and nine because outcomes could not

Table III. Perinatal outcome in subjects with unexplained elevated maternal serum AFP levels ≥2.5 multiples of the median compared with matched controls by matched-pair analysis (McNemar's test)

	All adverse outcomes	*Major congenital malformations*	*Preterm labor*	*Preterm rupture of membranes*	*IUGR*	*Abruptio placentae*	*Pregnancy-induced hypertension*	*Spontaneous abortion*
Subjects (n = 72)	28 (38.9%) p = 0.5	2 (2.8%) p = 0.99	8 (11.1%) p = 0.48	6 (8.3%) p = 0.75	6 (8.3%) p = 0.125	2 (2.8%) p = 0.625	9 (12.5%) p = 0.99	4 (5.6%) p = 0.125
Controls (n = 72)	23 (31.9%)	1 (1.4%)	12 (16.7%)	4 (5.6%)	1 (1.4%)	2 (2.8%)	10 (13.41%)	0

Table IV. Perinatal outcome in subjects (maternal serum AFP levels ≥2.5 multiples of the median) with risk factors and without risk factors compared with matched controls by matched-pair analysis (McNemar's test)

	All adverse outcomes	*Major congenital malformations*	*Preterm labor*	*Preterm rupture of membranes*	*IUGR*	*Abruptio placentae*	*Pregnancy-induced hypertension*	*Spontaneous abortion*
Subjects with risk factors (n = 36)	14 (38.9%) p = 0.359	2 (5.6%) p = 0.5	3 (8.3%) p = 0.34	1 (2.8%) p = 1.0	6 (16.7%) p = 0.125	1 (2.8%) p = 1.0	6 (16.7%) p = 0.38	1 (2.83%)
Controls with risk factors (n = 36)	11 (30.6%)	1 (2.8%)	7 (19.4%)	1 (2.8%)	1 (2.8%)	1 (2.8%)	3 (8.3%)	0
Subjects without risk factors (n = 36)	14 (38.9%) p = 0.6	0	5 (13.9%) p = 0.73	5 (13.9%) p = 0.73	0	1 (2.8%) p = 1.0	3 (8.3%) p = 0.34	3 (8.3%) p = 0.25
Controls without risk factors (n = 36)	12 (33.3%)	0	5 (13.9%)	3 (8.3%)	0	1 (2.8%)	7 (19.4%)	0

be obtained. The mean age of the remaining 72 patients was 24.9 years (range 15 to 37 years). Sixty-four (89%) of subjects were black; eight (11%) were white. Forty-six (64%) were multiparous. Unequivocal risk factors (Table I) were documented in 36 (50%) of the 72 subjects with unexplained elevated maternal serum AFP levels. The mean of the multiples of the median values in the control group was 1.16 ± 0.48 (SEM), indicating that the control group's maternal serum AFP values were not skewed high or low.

Pregnancy outcome data are summarized in Table III. Adverse perinatal outcome occurred in 38.9% (28 of 72) of subjects with unexplained elevated levels ≥2.5 multiples of the median, compared with 31.9% (23 of 72) of controls with normal levels (p = 0.5). Further, no statistically significant difference was found when subjects and controls were compared for any specific outcome (Table III). No stillbirths occurred in either the subject or control group.

To determine whether identifying Hollister risk factors helped predict perinatal outcome in patients with unexplained elevated maternal serum AFP levels, subjects with unexplained elevated levels and risk factors were compared with matched controls having normal levels and risk factors (Table IV). No statistically significant difference in adverse outcomes was observed. Similarly, comparing patients with unexplained elevated levels but no risk factors with matched controls with both normal levels and no risk factors again yielded no statistically significant difference with respect to adverse perinatal outcome (Table IV).

Additional analysis was performed comparing those subjects with maternal serum AFP value ≥3.0 multiples of the median with matched controls (Table V). Thirty-three subjects (45.8%) had values ≥3.0 multiples of the median. Adverse perinatal outcome occurred in 16 (48.5%) of these subjects, compared with 12 (36.4%) controls (p = 0.42). Again, no statistically significant difference for any specific outcome was found when analyzed according to presence or absence of risk factors (Table VI).

Finally, verifying the high-risk nature of our population is the observation that the frequency of adverse outcome in controls with risk factors was similar to that in controls without risk factors (30.6% vs 33.3%, Table IV). This seems to suggest that in our population preexisting risk factors do not identify patients at high risk. However, six of these 36 controls without risk factors were young primigravid women with pregnancy-induced hypertension. In our population pregnancy-in-

Table V. Perinatal outcome in subjects with unexplained elevated maternal serum AFP levels ≥3.0 multiples of the median compared with matched controls by matched-pair analysis (McNemar's test)

	All adverse outcomes	*Major congenital malformations*	*Preterm labor*	*Preterm rupture of membranes*	*IUGR*	*Abruptio placentae*	*Pregnancy-induced hypertension*	*Spontaneous abortion*
Subjects (n = 33)	16 (48.5%) p = 0.42	1 (3.0%) p = 0.5	6 (18.2%) p = 1.0	3 (9.1%) p = 1.0	4 (12.1%) p = 0.125	1 (3.0%) p = 0.5	5 (15.2%) p = 0.75	3 (9.1%) p = 0.25
Controls (n = 33)	12 (36.4%)	1 (3.0%)	5 (15.2%)	2 (6.1%)	1 (3.0%)	1 (3.0%)	5 (15.2%)	0

Table VI. Perinatal outcome in subjects (maternal serum AFP ≥3.0 multiples of the median with risk factors and without risk factors compared with matched controls by matched-pair analysis (McNemar's test)

	All adverse outcomes	*Major congenital malformations*	*Preterm labor*	*Preterm rupture of membranes*	*IUGR*	*Abruptio placentae*	*Pregnancy-induced hypertension*	*Spontaneous abortion*
Subjects with risk factors (n = 20)	14 (38.9%) p = 0.359	1 (5.0%) p = 1.0	3 (15.0%) p = 1.0	1 (5.0%) p = 1.0	4 (20.0%) p = 0.38	1 (5.0%) p = 1.0	3 (15.0%) p = 0.63	1 (5.0%)
Controls with risk factors (n = 20)	11 (30.6%)	1 (5.0%)	3 (15.0%)	1 (5.0%)	1 (5.0%)	1 (5.0%)	1 (5.0%)	0
Subjects without risk factors (n = 13)	7 (53.9%) p = 0.5	0	3 (23.1%) p = 1.0	2 (15.4%) p = 1.0	0	0	2 (15.4%) p = 0.69	2 (15.4%) p = 0.5
Controls without risk factors (n = 13)	6 (46.2%)	0	2 (15.4%)	1 (7.7%)	0	0	4 (30.8%)	0

duced hypertension is very common, occurring in 19.4% of controls without risk factors in this study. If young primigravid women without risk factors and pregnancy-induced hypertension are excluded from analysis, controls with Hollister risk factors indeed show a trend toward more adverse outcomes (13.2%) than controls without risk factors (7.9%).

Comment

Our matched-pair analysis failed to confirm the observations of others that patients with unexplained elevated maternal serum AFP have an increased risk of adverse perinatal outcome regardless of whether a cutoff of ≥2.5 or ≥3.0 multiples of the median was used. We realize that the number of subjects in this study is relatively small, limiting the power. To achieve a power of 0.80 and a significance level of 0.05, a sample size of 350 subjects would be required to detect a difference of 10.5% in adverse outcomes. However, our findings suggest that the clinical significance of unexplained elevated maternal serum AFP levels in an urban, indigent population having an a priori increased risk for adverse perinatal outcome needs to be reconsidered.

When subjects were subgrouped according to presence or absence of risk factors and compared with controls (matched for the presence or absence of risk factors), no predictive value for elevated maternal serum AFP was found. IUGR occurred in six (16.7%) subjects with unexplained elevated levels and one (1.4%) control; this difference is not statistically significant (p = 0.125). A larger sample size may have demonstrated significance. However, all subjects with IUGR had preexisting factors that placed them at high risk for such an outcome (e.g., significant drug and alcohol use, n = 4; prior neonatal death, n = 2; hypertension, n = 1).

In our subjects with preexisting risk factors, an unexplained elevated maternal serum AFP level did not further define a subpopulation at even higher risk. Furthermore, no difference in adverse perinatal outcome was observed when subjects with unexplained elevated levels and no Hollister risk factors were compared with controls with normal levels and no Hollister risk factors. Irrespective of whether women actually had preexisting Hollister risk factors, maternal serum AFP did not appear to have additional predictive value for adverse perinatal outcome.

In summary, the association between unexplained

maternal serum AFP levels and adverse perinatal outcome may not apply to all obstetric populations. Our study differs from others[1-6] in that it involved an indigent urban population that is already known to be at increased risk for adverse perinatal outcome. Unexplained elevated maternal serum AFP does not appear to have additional predictive value in this population. We therefore suggest further studies from institutions whose populations are similar to ours.

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Pelvic inflammatory disease: Trends in hospitalizations and office visits, 1979 through 1988

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OBJECTIVES: We attempted to assess trends in pelvic inflammatory disease occurrence and to describe current antibiotic treatment and use of surgical procedures for pelvic inflammatory disease in the United States.
STUDY DESIGN: Analyses of hospitalizations according to the National Center for Health Statistics, National Hospital Discharge Survey for 1979 to 1988, and of office visits to private physicians from the National Disease and Therapeutic Index for 1979 to 1989 were done.
RESULTS: From 1979 to 1988, a mean of 181,700 women aged 15 to 44 years were hospitalized each year for acute pelvic inflammatory disease (3.03/1000 women) and 94,400 for chronic pelvic inflammatory disease (0.90/1000), and nearly 400,000 first visits for pelvic inflammatory disease were made each year to private physicians' offices (7.2/1000 women). Mean visit and hospitalization rates for acute pelvic inflammatory disease were highest for women aged 20 to 24 years and for other-than-white women. By 1987 to 1988, however, pelvic inflammatory disease hospitalization rates were highest for teenagers. Surgery was performed during 42% of hospitalizations for acute pelvic inflammatory disease and 90% of hospitalizations for chronic pelvic inflammatory disease. Over this time period, hospitalization rates for acute pelvic inflammatory disease decreased by 36% while office visit rates remained unchanged.
CONCLUSION: This decrease in hospitalizations for pelvic inflammatory disease may indicate a true decrease in its incidence, changes in physician hospitalization practices, or a shift in the spectrum of severity of pelvic inflammatory disease. (Am J Obstet Gynecol 1992;166:983-90.)

Key words: Pelvic inflammatory disease, salpingitis, epidemiology, surveillance

Pelvic inflammatory disease is a common serious complication of sexually acquired infection[1-3] and can cause tubal damage leading to infertility, ectopic pregnancy, and chronic pelvic pain.[4, 5] The total economic cost of pelvic inflammatory disease was estimated at $4.2 billion in 1990.[6] Because pelvic inflammatory disease is the most important sequela of cervical infection caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, data on its occurrence are needed both to identify those at risk of sequelae and to evaluate public health initiatives to reduce occurrence of these infections.

Pelvic inflammatory disease is not a reportable condition, however, and data on the actual number of cases occurring in the United States are unavailable. Therefore we analyzed recent trends in hospitalizations and visits to private, office-based physicians for pelvic inflammatory disease in an attempt to understand recent trends in occurrence. Additionally, we examined characteristics of and trends in management of patients with pelvic inflammatory disease, including antibiotic therapy, surgical treatment, and duration of hospitalization.

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Material and methods

Data on hospitalizations for pelvic inflammatory disease were obtained from the National Hospital Discharge Survey (NHDS) conducted annually by the National Center for Health Statistics. The NHDS abstracts demographic data, final diagnoses, and procedures performed from a sample of medical records from approximately 400 nonfederal, short-stay hospitals representing all 50 states and the District of Columbia. More than 200,000 medical records are sampled each year and the data are weighted to represent approximately 30 million hospital discharges per year.

Data from the NHDS were analyzed from 1979 through 1988, the period during which the *International Classification of Diseases, ninth revision, Clinical Modification*[7] (ICD-9) was used to code discharge diagnoses. Patient discharges were included as hospitalizations for pelvic inflammatory disease if one of the following ICD-9 codes was listed as one of up to seven final diagnoses: salpingitis and oophoritis (614.0 to 614.2, 098.17, 098.37), parametritis and pelvic peritonitis (614.3 to 614.5, 0.98.86), pelvic inflammatory disease (614.7 to 614.9, 098.10, 098.30, 098.39), and inflammatory disease of the uterus, except cervix

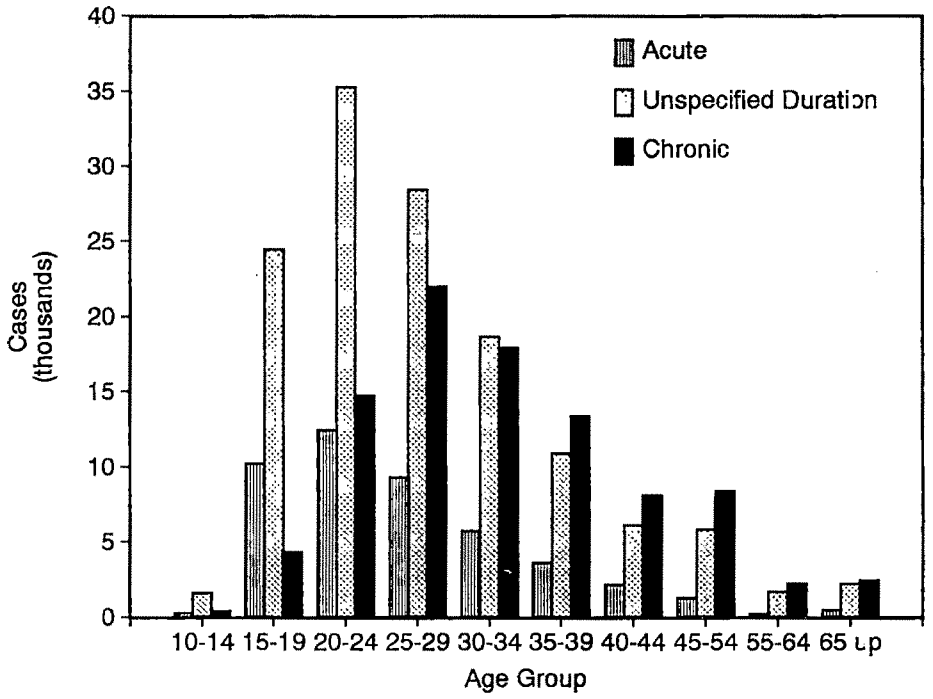


Fig. 1. Average annual number of hospitalizations for pelvic inflammatory disease in United States from 1979 to 1988. Age distribution of cases by disease duration as classified by ICD-9 disease codes (see text).

(615.0, 615.1, 615.9, 098.16, 098.36). These diagnostic classifications specifically exclude complications of abortion or childbirth. Within some diagnostic categories, cases are coded as being of acute, chronic, or unspecified duration; in some categories duration cannot be specified. To assess the significance of the specified duration of pelvic inflammatory disease, hospitalizations were initially classified as acute if at least one acute pelvic inflammatory disease code was listed, as chronic if at least one chronic (but no acute) code was listed, and as unspecified if neither acute nor chronic codes were listed. These results were used to guide subsequent analyses.

The NHDS lists up to four procedures for each hospitalization. We classified surgical procedures that seemed likely to be related to pelvic inflammatory disease and involved the abdominal or pelvic organs as being primarily diagnostic, therapeutic (without organ removal), or for organ removal.

Data on outpatient visits for pelvic inflammatory disease were obtained from the National Disease and Therapeutic Index for the years 1979 to 1989. This index is a commercially conducted (IMS America) survey of patient encounters with physicians in office-based, private practices in the United States. Approximately 2130 physicians are surveyed quarterly to represent >200,000 eligible physicians in 19 primary care specialties. Physicians who are selected report on all patient encounters during one 48-hour period each quarter. National estimates of patient-physician encounters are projected from this sample. Diagnoses were coded with the *International Classification of Diseases, seventh revision*[8] (ICD-7) through 1986 and with ICD-9 from 1987 to 1989. A visit was considered to represent pelvic inflammatory disease if an ICD-9 code indicating either acute or unspecified-duration pelvic inflammatory disease (same as for the National Hospital Discharge Survey) was listed for the years 1987 to 1989 or a corresponding ICD-7 code (salpingitis or oophoritis, 6220, 6230, 6240; pelvic inflammatory disease and parametritis 6262; endometritis, 6301) was listed for the years 1979 to 1986. Chronic pelvic inflammatory disease was rarely reported.

Of the six categories of patient-physician encounters reported in the National Disease and Therapeutic Index (office visits, house calls, hospital visits, phone calls, nursing home visits, and other), only office visits were included in this analysis. Demographic data, antibiotic and other drug therapy provided, and whether the visit was the initial one for that patient and condition are recorded for each visit. For most analyses, data are presented for the 10-year period 1979 to 1988 to be compatible with the hospitalization data and allow the use of 5-year averages.

Annual age, race, marital status, and region-specific hospitalization rates (National Hospital Discharge Survey) and physician visit rates (National Disease and Therapeutic Index) were calculated using Bureau of the Census population estimates.[9-12] Race was categorized as white or other-than-white. Mean case totals for

Table I. Average annual numbers and rates of hospitalizations for pelvic inflammatory disease among United States women aged 15 to 44 years, 1979 to 1988

Characteristic	Acute			Chronic		
	Cases*	Rate†	SE‡	Cases	Rate	SE
Women aged 15 to 44 yr	167,800	3.03	0.05	80,750	1.46	0.03
Age group (yr)						
15-19	34,780	3.77	0.08	4,360	0.47	0.02
20-24	47,800	4.49	0.09	14,800	1.39	0.04
25-29	37,830	3.51	0.08	22,050	2.05	0.05
30-34	24,510	2.48	0.06	17,950	1.81	0.05
35-39	14,560	1.70	0.05	13,430	1.56	0.05
40-44	8,350	1.20	0.04	8,160	1.18	0.04
Marital status§						
Single	75,490	3.84	0.07	19,780	1.01	0.02
Married	68,750	2.37	0.04	47,130	1.62	0.04
Widowed	900	1.86	‖	820	1.69	*‖
Divorced	15,820	3.53	0.10	9,570	2.14	0.07
Separated	6,850	3.86	0.14	3,460	1.94	0.09
Race§						
White	110,200	2.37	0.04	57,650	1.24	0.03
Other-than-white	57,640	6.49	0.13	23,100	2.60	0.07
Region						
Northeast	27,220	2.33	0.11	12,410	1.06	0.07
Midwest	40,780	2.95	0.13	19,560	1.41	0.08
South	72,400	3.83	0.14	32,840	1.74	0.08
West	27,440	2.45	0.12	15,940	1.42	0.09

*Average annual number of cases in years 1979 to 1988.

†Rate per 1000 women and based on average annual cases and average population (except for marital status, which is based on 1984 population estimates).

‡Standard errors of rate estimates, expressed as cases per 1000 women.

§Cases with missing information (14% of cases for marital status, 7% for race) were prorated to distribution of known cases.

‖Estimates for which valid confidence limits could not be calculated. These point estimates should be interpreted with caution.

2- or 5-year time periods were used to improve the precision of some estimates.

χ^2 testing, incorporating a correction factor based on sampling weights and correlations between years, was used to test differences between proportions. Weighted least-squares analysis (using inverse of variance as weights) was used to test time trends . A *t* test was used to test differences between means. Standard error estimates were provided by each survey.

Results

Rates and trends in hospitalizations for pelvic inflammatory disease. Between 1979 and 1988, a mean of 276,100 hospitalizations for pelvic inflammatory disease occurred each year; of these, a mean of 46,100 (17%) were classified as being for acute illness, 94,400 (34%) for chronic illness, and 135,600 (49%) for illness of unspecified duration. Women hospitalized for chronic disease were distinctly older than women hospitalized for acute and unspecified pelvic inflammatory disease (Fig. 1). For hospitalizations where marital status was known (86%), 58% of women hospitalized for chronic pelvic inflammatory disease, compared with 35% for acute and 43% for disease of unspecified duration, were married ($p < 0.01$). Surgery was performed during 90% of hospitalizations for chronic pelvic inflammatory disease, compared with 34% for acute disease and 41% for disease of unspecified duration ($p < 0.01$). The diagnosis of chronic pelvic inflammatory disease thus appeared to represent a distinctly different clinical syndrome occurring in a different subset of women. Therefore for subsequent analyses hospitalizations for acute or unspecified-duration pelvic inflammatory disease were grouped together as acute disease and analyzed separately from those for chronic disease. Women from 15 to 44 years of age accounted for 92% of hospitalizations for acute and 86% of hospitalizations for chronic pelvic inflammatory disease; subsequent analyses were restricted to women in this age group.

The mean annual rate of hospitalization for acute pelvic inflammatory disease was highest for women 15 to 29 years of age (Table I); in contrast, rates for chronic pelvic inflammatory disease were highest for women 25 to 39 years old. Mean annual hospitalization rates were higher for women of other-than-white race than for women of white race for both acute (rate ratio 2.74, 95% confidence interval 2.60 to 2.89) and chronic pelvic inflammatory disease (2.10, 1.97 to 2.23). The mean ages and age distributions did not vary by racial group for acute or chronic pelvic inflammatory disease. For acute disease, rates were higher for women who were

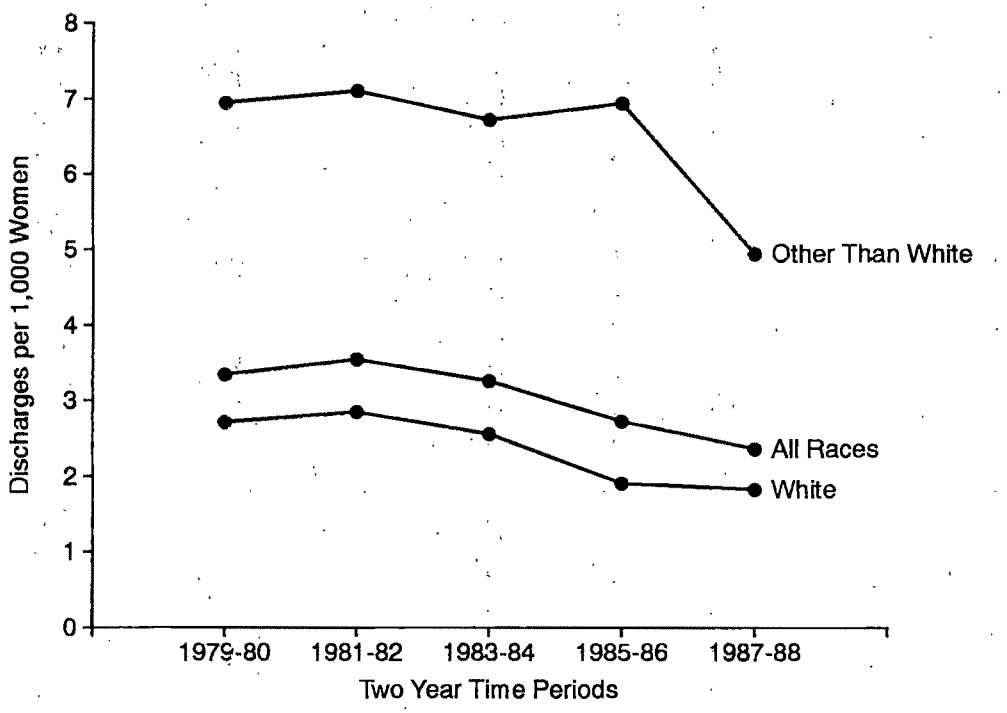


Fig. 2. Trends in hospitalizations for acute pelvic inflammatory disease among white and other-than-white women in United States from 1979 to 1988. Acute pelvic inflammatory disease refers to ICD-9 codes designating acute or unspecified duration disease (see text). Rates are presented as 2-year means.

single, separated, or divorced than for women who were married or widowed ($p < 0.05$). For both acute and chronic pelvic inflammatory disease, rates were highest in the South, lowest in the Northeast, and intermediate in the Midwest and West ($p < 0.05$).

A decrease in the incidence of hospitalization for acute pelvic inflammatory disease in the United States began in about 1983 and continued through 1988 (Fig. 2). Overall, rates decreased 36% from 1979 to 1988, from 3.47 to 2.23 per 1000 women. Over the decade, rates decreased similarly for women of both racial groups; hospitalization for acute pelvic inflammatory disease was 2.7 times more common for women of other races than for white women in 1987 to 1988, essentially the same as in 1979 to 1980 (2.6 times higher).

Hospitalization rates also decreased for all age groups from 15 to 44 years of age (Fig. 3). Rates decreased least for 15 to 19 year olds (10%); as a result, 1987 to 1988 hospitalization rates were highest for this age group. Hospitalization rates for acute pelvic inflammatory disease decreased in all four geographic regions.

During this time period, the hospitalization rate for chronic pelvic inflammatory disease decreased by 40%, from 1.82 in 1979 to 1980 to 1.09 in 1987 to 1988. As with acute pelvic inflammatory disease, rates decreased in all age groups and regions and for both racial groups.

Rates and trends in office visits for pelvic inflammatory disease. Between 1979 and 1988, a mean of 1.2 million visits to private physicians' offices were made each year for acute pelvic inflammatory disease. A mean of 420,000 visits (34%) each year were initial visits for pelvic inflammatory disease. Of these, 95% were by women 15 to 44 years of age, a mean annual rate of 7.2 visits per 1000 women (95% confidence interval 7.15 to 7.25). The rate of initial office visits for acute pelvic inflammatory disease changed little over this time period (Table II).

Most visits for pelvic inflammatory disease were to obstetrician-gynecologists (45%) or general and family practitioners (27%); the remainder were seen by physicians in a variety of specialties. This pattern did not change over the study period.

The age distribution of annual visit rates from the National Disease and Therapeutic Index was similar to the age distribution of hospitalizations for acute pelvic inflammatory disease (Tables I and II). Visit rates for white women were lower than for women of other races, although the rate ratio comparing other-than-white women with white women for initial office visits (1.6, 95% confidence interval 1.1 to 2.1) was substantially lower than the comparable rate ratio for hospitalizations for acute pelvic inflammatory disease. To evaluate change by racial grouping, 4-year means were calculated for the years 1982 to 1985 and 1986 to 1989. Visit rates changed by <0.2 visits per 1000 women per

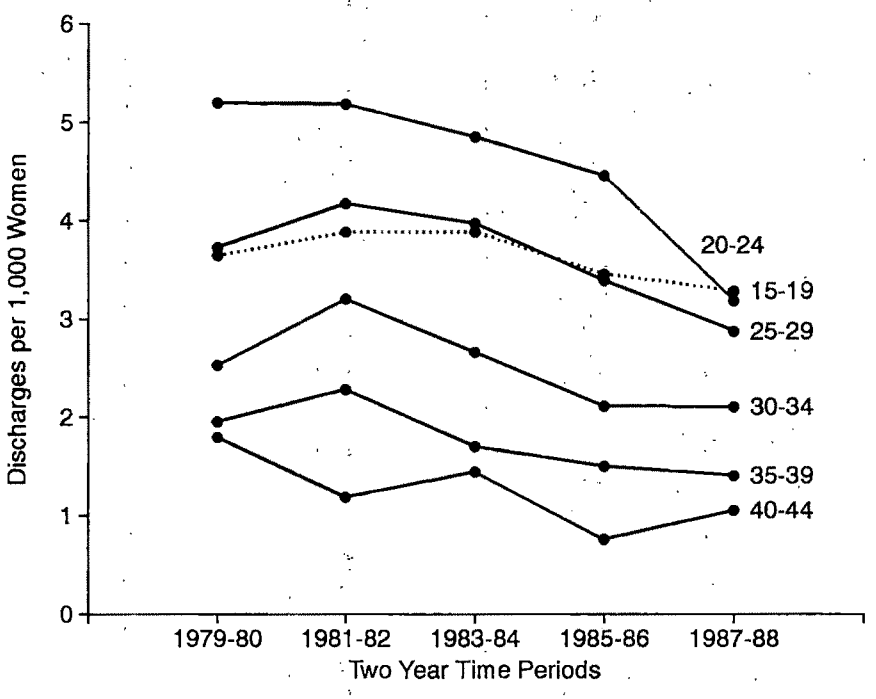


Fig. 3. Trends in hospitalizations for acute pelvic inflammatory disease in United States from 1979 to 1988, by 5-year age groups. Acute pelvic inflammatory disease refers to ICD-9 codes designating acute or unspecified-duration disease (see text). Rates are presented as 2-year means.

year for women of both racial groups between these two time periods (data not shown). As for hospitalizations, pelvic inflammatory disease visit rates were highest in the South.

Surgical and diagnostic procedures for pelvic inflammatory disease. Surgery was performed during 42% of hospitalizations for acute and 90% of hospitalizations for chronic pelvic inflammatory disease ($p < 0.01$). Among patients with acute disease, the proportion undergoing surgery increased steadily with increasing age, from 30% of 15- to 19-year-olds to 66% of 40- to 44-year-olds ($p < 0.01$). Surgery was more common for white women (44%) than for women of other races (37%, $p < 0.01$). The rate of surgery varied little by region for either acute or chronic pelvic inflammatory disease.

Of patients with acute disease undergoing surgery 36% underwent a procedure involving organ removal, 29% a therapeutic procedure not involving organ removal, and 17% a diagnostic procedure only; the other 19% had a surgical procedure that appeared unrelated to pelvic inflammatory disease. Among patients with chronic disease undergoing surgery, 63% underwent an organ removal procedure ($p < 0.01$, comparison of acute and chronic pelvic inflammatory disease). Commonly performed surgical procedures included lysis of peritoneal adhesions, hysterectomy, salpingo-oophorectomy, laparoscopy, and curettage.

Laparoscopy, often considered the gold standard test for diagnosing pelvic inflammatory disease, was performed for 12% of patients with acute and 19% of patients with chronic disease between 1979 and 1988. These proportions changed little over the study period. The most notable change in procedures used to diagnose or treat pelvic inflammatory disease over this time period occurred for pelvic ultrasonography, use of which increased from 2% of hospitalizations for acute pelvic inflammatory disease in 1979 to 19% of hospitalizations in 1988 ($p < 0.01$). Ultrasonography was used more commonly for younger women (14% in the 15- to 19-year age group compared with 3% in the 40- to 44-year age group in 1984 to 1988). Use of pelvic ultrasonography also varied among regions; in 1987 to 1988, it was used for 33% of hospitalizations in the Northeast, 19% in the Midwest and West, and 11% in the South ($p < 0.05$). Ultrasonography was used infrequently for chronic versus acute pelvic inflammatory disease (3% vs 9%, 1979 to 1988, $p < 0.01$).

Hospital stay and payment method for pelvic inflammatory disease. The mean length of hospitalization over the 10-year period was 5.3 days (95% confidence interval 4.1 to 6.5) for acute and 5.9 days (3.9 to 7.9) for chronic pelvic inflammatory disease. The mean hospital stay did not change significantly over the study period. For both acute and chronic disease, length of hospital stay increased with increasing age, from 4.7 days in the 15- to 19-year age group to 7.2 days in the 40- to 44-year age group for acute pelvic inflammatory

Table II. Average annual numbers and rates of visits to private physicians' offices for acute pelvic inflammatory disease by United States women aged 15 to 44 years for 1979 to 1983 and 1984 to 1988*

Characteristic	1979-1983			1984-1988		
	Visits	Rate†	SE‡	Visits	Rate	SE
Women aged 15 to 44 yr	376,450	7.1	0.4	419,530	7.3	0.4
Age groups (yr)						
15-19	57,650	5.8	§	59,810	6.6	§
20-24	111,030	10.5	1.1	108,080	10.7	1.1
25-29	96,220	9.6	1.1	126,140	11.6	1.2
30-34	58,130	6.4	§	68,010	6.5	§
35-39	32,200	4.3	§	36,670	4.0	§
40-44	21,210	3.4	§	20,810	2.8	§
Race‖						
White				319,840	6.7	0.4
Other races				99,690	10.5	1.5
Region						
Northeast	51,350	4.5	§	44,960	3.8	§
Midwest	68,860	5.0	§	72,070	5.2	§
South	193,390	10.7	0.9	205,700	10.5	0.8
West	62,850	5.9	§	96,800	8.2	§

*Initial office visits for acute pelvic inflammatory disease. "Acute" refers to ICD-7 and ICD-9 codes for acute or unspecified-duration pelvic inflammatory disease.

†Rate per 1000 women and based on average annual number of visits and average population.

‡Standard error of rate estimate, expressed as cases per 1000 women.

§Estimates for which valid confidence limits could not be calculated. These point estimates should be interpreted with caution.

‖Race data were not available for 1979 to 1981. For 1984 to 1988, cases with missing racial information (1.5%) were prorated to distribution of known cases.

disease ($p < 0.05$ for trend). Hospital stays did not differ among regions or between racial groups.

The cost of both acute and chronic pelvic inflammatory disease hospitalization was most commonly covered by private insurance. For acute disease, 59% of hospitalizations were covered by private insurance, 26% by public payment methods, and 15% by other means (no charge, self-pay, other). Public payment was more common for women of other-than-white race than for white women (36% and 19% for acute pelvic inflammatory disease) and for younger women than for older women (28% for 15- to 19-year-olds and 14% for 40- to 44-year-olds). Payment patterns changed little over the study period.

Antibiotic therapy for pelvic inflammatory disease. Treatment with at least one antimicrobial drug was recorded for 87% of office visits for pelvic inflammatory disease from 1979 to 1989. Of these, at only half (54%) of visits did the woman receive a drug effective against *C. trachomatis* (tetracycline, erythromycin, or clindamycin) and only 4% were treated with a two-antibiotic regimen that included a drug active against *C. trachomatis*. The proportion of visits at which a drug effective against *C. trachomatis* was prescribed increased over the study period, however, from 32% in 1979 to 74% in 1989 ($p < 0.01$). During 1989, 21% of patients received an appropriate two-antibiotic combination.

Comment

These data suggest that the number of patients hospitalized for pelvic inflammatory disease decreased during the 1980s, whereas the number of patients being seen in physicians' offices was stable. The increased risk for women of other-than-white race remained essentially unchanged. Hospitalization rates decreased least for teenage girls who now have the highest rate of hospitalization. The cost of pelvic inflammatory disease, both in economic terms to society and in its adverse effects on the reproductive health of women, was reflected by the high frequency of surgery during hospitalizations. Trends in antibiotic treatment for ambulatory patients with pelvic inflammatory disease suggest that physicians' understanding of this syndrome has increased but substantial room for improvement remains.

These data have several potentially important limitations. First, both surveys have limited precision. This particularly limits the ability to adjust for potentially confounding variables. Second, the data may not be representative of all pelvic inflammatory disease occurring in the United States. Patients who require hospitalization may not be typical of all patients. In addition, the National Disease and Therapeutic Index, which samples only office-based private physicians, fails to track patients seen in settings such as public clinics and emergency rooms. The ratio between rates for

other-than-white and white women may be lower in the National Disease and Therapeutic Index than in the NHDS because other-than-white patients are less likely to consult a private physician for symptoms of pelvic inflammatory disease. Third, neither survey uses standard case definitions. This is a concern given the established poor positive predictive value of a clinical diagnosis.[5, 13] The diagnosis recorded at hospital discharge may be more accurate than the reported 65% positive predictive value of a clinical diagnosis, however, because of the frequent use of surgical exploration, laparoscopy, and pelvic ultrasonography.

The coding system used, the *International Classification of Diseases,* also has limitations. Whereas duration can be specified for the organ-specific diagnoses (e.g., salpingitis and endometritis), duration cannot be specified for the diagnosis most compatible with the diagnostic certainty possible in most clinical settings. Thus duration is unspecified for nearly half of pelvic inflammatory disease hospitalizations.

In spite of these problems, ICD codes for acute (acute and unspecified combined) and chronic pelvic inflammatory disease appear to measure different conditions, possibly different stages in the disease process. This conclusion is supported by the differences in age, marital status, and frequency of surgery between these two categories. Hospitalizations for chronic pelvic inflammatory disease probably represent sequelae of prior episodes of acute pelvic inflammatory disease, rather than complications of recently acquired cervical infection. As such they are an important measure of the cost of pelvic inflammatory disease for individual women and for the health care system but are unlikely to be useful for tracking the effectiveness of current prevention efforts. The combination of acute and unspecified pelvic inflammatory disease probably contains some discharges for chronic conditions but in our opinion represented the best available definition to estimate the number of and trends in cases of recently acquired infection.

The decrease in hospitalizations for pelvic inflammatory disease in the United States may indicate a decrease in the occurrence of the disease, as has occurred in Sweden.[14] This would be encouraging, especially in the face of recently reported increases in hospitalizations for pelvic inflammatory disease in England.[15] The decreasing incidence of gonorrhea since the late 1970s[16] may have led to a lower incidence of pelvic inflammatory disease.

Alternatively, the divergent trends for hospitalizations and office visits may indicate that fewer patients with pelvic inflammatory disease are being hospitalized. It is possible that pelvic inflammatory disease caused by *N. gonorrhoeae,* which is usually more clinically severe,[17] has decreased more than has pelvic inflammatory disease caused by *C. trachomatis.* This could have led to fewer patients being diagnosed with pelvic inflammatory disease or to a decrease in the proportion of patients who require hospitalization. Physicians may also be less likely to hospitalize women for treatment of recognized pelvic inflammatory disease. Hospitalizations for all conditions have decreased over this time period,[18] suggesting that changes in practice patterns may be at least partly responsible for the decrease in hospitalizations for pelvic inflammatory disease.

Several previous studies have indicated that pelvic inflammatory disease is often treated suboptimally.[19-21] Improvement has occurred as awareness of the importance of *C. trachomatis* has increased. However, according to current recommendations,[6] the majority of women are still treated suboptimally.

Prevention of pelvic inflammatory disease and its sequelae are important reasons for public health efforts to reduce the incidence of genital infection caused by *N. gonorrhoeae* and *C. trachomatis.* Accurate surveillance of pelvic inflammatory disease is needed to evaluate these programs. These data provide the best measures of occurrence in the United States. Although they offer important insights into the burden of pelvic inflammatory disease on the health care system and on the individual, important questions remain unanswered. Additional data about the epidemiology of pelvic inflammatory disease in the United States are needed. Surveillance could be improved by including cases seen in public clinics and emergency rooms, by obtaining bacteriologic data from detected cases, and by standardizing case definitions. Studies should assess the frequency and importance of clinically unrecognized, or silent, pelvic inflammatory disease. The culmination of these efforts will enable us to properly assess the effectiveness of our prevention efforts.

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Mimicking low amniotic pressure by chronic pharyngeal drainage does not impair lung development in fetal sheep

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OBJECTIVE: The etiology of oligohydramnios-related pulmonary hypoplasia is not understood but is known to involve chronic lung liquid loss. We tested the hypothesis that low amniotic pressure in oligohydramnios disturbs the normal tracheal-amniotic pressure gradient to increase lung liquid loss and impair lung development.

STUDY DESIGN: Chronic pharyngeal catheterization with drainage to the exterior was used in 15 fetal sheep to mimic reduced amniotic pressure at the upper airway in the presence of normal amniotic fluid volume.

RESULTS: Pharyngeal pressures relative to amniotic pressures were negative in all drained fetuses (mean ± SE −3.0 ± 0.6 mm Hg), in contrast to positive pressures in controls (0.7 ± 0.1 mm Hg). There was no significant difference in lung weight or deoxyribonucleic acid relative to body weight, or in lung morphometry, between 10 fetuses drained for 10 to 21 days and their control cotwins.

CONCLUSION: Mimicking low amniotic pressure in the upper airway by chronic fetal pharyngeal drainage does not impair lung development in fetal sheep. (Am J Obstet Gynecol 1992;166:991-6.)

Key words: Pulmonary hypoplasia, oligohydramnios, low amniotic pressure, fetal lung development, fetal sheep

Pulmonary hypoplasia, found in 15% to 21% of perinatal necropsy specimens, remains a common cause of perinatal mortality.[1, 2] Whereas inhibition of fetal breathing movements and compression by intrathoracic space-occupying lesions are known causes of pulmonary hypoplasia in both human[1, 3] and animal studies,[4, 5] the most common cause is oligohydramnios.[1] Although removal of amniotic fluid has been shown to produce pulmonary hypoplasia in numerous animal studies,[6-10] the exact mechanism by which oligohydramnios inhibits lung growth is not understood.

The mechanism of oligohydramnios-related pulmonary hypoplasia is unlikely to involve inhibition of fetal breathing movements, because fetal breathing movements continue during oligohydramnios in animals[7, 11] and their incidence is no lower in human fetuses with oligohydramnios-related pulmonary hypoplasia compared both to those with oligohydramnios and normal lungs[12] and to normal controls.[13] Compression of the thoracic cavity is also an unlikely mechanism, because amniotic pressure in severe oligohydramnios is paradoxically low[11, 14, 15] and experimentally induced abdominal compression has no effect on lung development.[16]

Normally, the upper airways produce resistance to the egress of lung liquid, creating a standing tracheal pressure of 1.5 to 3.0 mm Hg above amniotic pressure.[7, 17, 18] As lung development is impaired when this resistance is bypassed in tracheostomy experiments,[19, 20] it has been suggested that lung liquid acts as an internal stent around which the lung grows.[6, 19] Loss of lung liquid from the airways into the amniotic cavity is implicated in oligohydramnios-related pulmonary hypoplasia by the following: (1) lung volume is reduced in chronic oligohydramnios,[8] (2) tracheal ligation in experimental studies prevents the adverse pulmonary effects of oligohydramnios,[6] and (3) human fetuses with renal agenesis do not develop pulmonary hypoplasia if laryngeal atresia is also present, as in Fraser's syndrome.[21, 22]

We recently hypothesized that the mechanism causing pulmonary hypoplasia in oligohydramnios is a reduction in amniotic pressure disturbing the normal tracheal-amniotic pressure gradient, thereby increasing

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lung liquid escape.[15] The aim of this study was to determine whether mimicking reduced amniotic pressure at the upper airway without creating oligohydramnios impaired fetal lung development.

Material and methods

Surgical procedure. Chronically instrumented fetal sheep were prepared as described previously[23] with standard procedures approved by the host institution and in accordance with United Kingdom legislation. Briefly, anesthesia was induced with thiopental (1 gm given intravenously) in 15 cross-bred ewes with twin pregnancies at 112 to 120 days' gestation and maintained with halothane (1.5% to 2.0% in oxygen). One horn of the gravid uterus was exposed at laparotomy and the fetal head was delivered through a uterine incision. Catheters were implanted into a fetal carotid artery, the fetal oropharynx, and the amniotic cavity. In six fetuses, a catheter was also placed into the trachea. Multiple fenestrations were fashioned at one end of a polyvinyl tube 60 cm long (inner diameter 5.5 mm), which was then fixed in the fetal oral cavity. This catheter was placed on the dorsal surface of the tongue with its tip approximately 3.5 cm posterior to the central incisors and lying at the extreme posterior margin of the tongue so as to prevent the tongue obstructing the connection between the catheter's lumen and the oropharynx. Its diameter occupied most of the cavity at this point. The lips and nares were sewn closed and sealed with cyanoacrylate glue. The pharyngeal drainage tube was filled with normal saline solution (0.9% sodium chloride), and the integrity of the pharyngeal seal isolating the pharyngeal from the amniotic cavity was confirmed. As leakage around the catheter subsequently developed in some, an additional seal was achieved in the last eight preparations by enveloping the snout in a rubber glove tied around the drainage tube. The fetus was replaced, the uterus was closed in two layers, and all catheters were exteriorized through the ewe's right flank. A collection bag, connected to the pharyngeal drain by a wide-bore polyvinyl tube 90 cm long was positioned approximately 40 cm beneath the floor of the cage. Drainage was commenced immediately. At least 5 days were allowed for postoperative recovery before recordings were made, during which time antibiotics were administered to ewe and fetus.

Catheters for fetal pharyngeal, tracheal, and amniotic pressure measurement were similarly placed for control readings in three fetuses (from three ewes at 118 to 120 days' gestation) whose upper airways were otherwise left intact.

Experimental protocol. After fetal pH and blood gas values had been shown to be normal (mean ± SEM, pH 7.35 ± 0.01, P_{CO_2} 43.1 ± 0.7 mm Hg, P_{O_2} 21.2 ± 0.9 mm Hg), pressures were measured with standard biomedical transducers attached to fluid-filled lines. Electronically subtracted pharyngeal-amniotic and tracheal-amniotic pressures were recorded for 1 hour every 2 to 3 days on a polygraph run at either 1.25 or 1.5 cm/min. The drainage bag was emptied every 2 to 3 days, and the equivalent volume of normal saline solution (0.9% sodium chloride) warmed to 38° to 39° C was infused intraamniotically. In addition, 2 ml of indigo carmine dye diluted 1:20 was added to the saline solution, so that the appearance of dye in the drainage bag gave an immediate indication of a break in the seal between the pharynx and the amniotic cavity. Dye was similarly instilled whenever the pharyngeal-amniotic pressure was > -0.5 mm Hg or when the volume of pharyngeal fluid in the drainage bag increased by >300 ml/day. Samples of the drained liquid were frozen for later viscometric analysis.

Lung specimens. The experiment was terminated on day 21 of drainage, when labor ensued, or when the dye indicated leakage. Animals were killed with pentobarbital (4 gm given intravenously), necropsy was performed, and both twins were delivered, towel dried, and weighed. Pulmonary variables were analyzed in those experiments with >10 days' drainage. The fetal tracheas were transected 0.5 cm above the carina and lung liquid was allowed to drain passively. The lungs were then removed, dissected, and weighed separately. The left main bronchus from each twin was cannulated and the left lung was inflated with buffered formalin under constant pressure (25 cm water for 30 minutes followed by 15 mm water for 11.5 hours). Five representative blocks approximately 2 mm thick were obtained from both lobes, and sections were prepared in standard fashion for morphometric study. Five fields of view per section were examined microscopically (magnification × 40) from each of five representative blocks, and the inner surface of each airspace within a 1 cm^2 sample was traced by light pen on a morphometry tablet. The mean number of airspaces counted was 57.9 ± 3.7.

For deoxyribonucleic acid (DNA) estimation, portions of right lung approximately 2 cm in diameter were harvested from each lobe, weighed (range 10 to 25 gm), and stored at −20° C. Samples were homogenized in an equivalent weight of Hepes buffer with a Polytron PCU-2 for 5 minutes and sonicated for 6 minutes, demonstrated in preliminary experiments as necessary for optimal DNA extraction. DNA was measured in duplicate as DNA/4,6-diamidino-2-phenylindole-2-hydrochloride complexes with a fluorometric microassay.[24] Laboratory specimens were coded to ensure that the DNA analysis and lung morphometry were performed blind. Two preparations with light microscopic signs of autolysis were excluded.

Analysis. In control and pharyngeally drained fe-

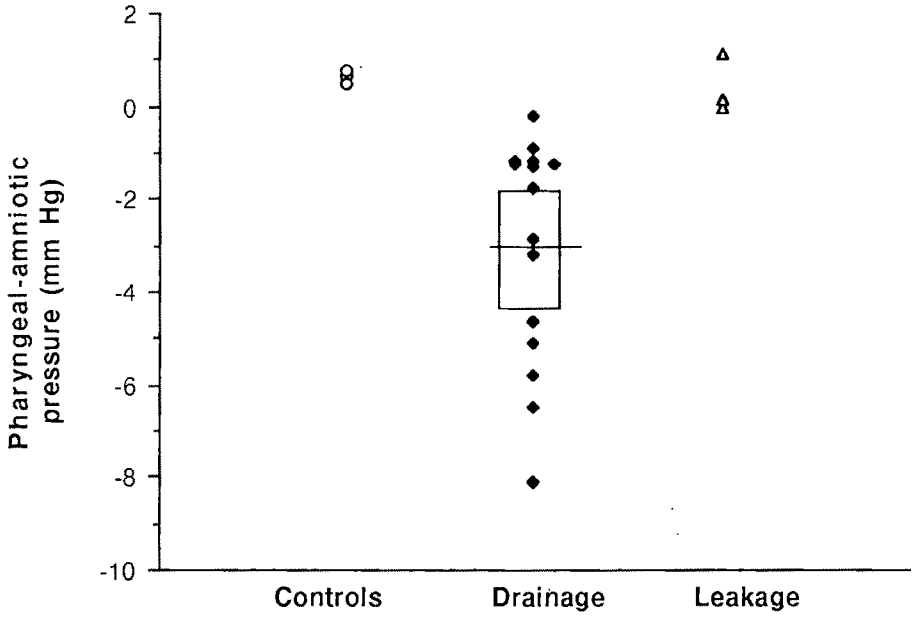


Fig. 1. Pharyngeal-amniotic pressures in three control fetuses (0) and in 15 fetuses that underwent chronic nasopharyngeal drainage (♦). *Box* indicates mean and 95% confidence intervals. Also shown are values from three drained fetuses taken on day mouth leakage was detected (△).

tuses, each minute of the pressure trace was examined for negative pressure fluctuations consistent with fetal breathing, gasping, or swallowing activity. Such minutes were excluded and point estimates of pressure were obtained at the midpoint of each minute, and a mean per hour was calculated. After exclusion of recordings obtained within 24 hours of labor or detection of mouth leakage, mean pharyngeal-amniotic pressures per drained animal were derived from a median of three recordings (range 2 to 7), and a pooled mean was calculated from the individual means per animal. Mean tracheal-amniotic pressures were similarly derived.

Viscosity was measured at 37° C with a rotating cup viscometer on a single specimen collected between days 8 and 10 of drainage from 10 fetuses. Values were then compared with those obtained in lung liquid specimens from previous experiments and stored at our institution involving chronic fetal tracheal drainage; these animals had been similarly instrumented, and the specimens were collected at comparable gestations and duration of drainage. Parametric statistics were used only where histograms confirmed a normal distribution. Pulmonary variables in the experimental twin were compared with those of the control twins by paired *t* testing; a Bonferroni correction for multiple comparisons was applied, so that differences <0.008 (0.05/6) were considered significant.

Results

Negative pharyngeal-amniotic pressures were achieved in all 15 drained fetuses, as shown in Fig. 1. Mean pharyngeal-amniotic (-3.0 ± 0.6 mm Hg) was significantly lower than 0 ($p < 0.001$) and significantly lower than that in controls (0.7 ± 0.1 mm Hg, $p = 0.02$).

The experimental period lasted ≥10 days in 10 drained animals: Two were killed on day 21, five went into labor, and mouth leakage occurred in three. Mean pharyngeal-amniotic pressure in these 10 experimental fetuses also remained significantly negative (-2.7 ± 0.6 mm Hg, $p = 0.001$) throughout the experimental period. In contrast, mean pharyngeal-amniotic pressures on the day of detection of mouth leakage was not significantly different from 0 (0.4 ± 0.4 mm Hg). The mean volume of fluid drained per day in the 10 preparations lasting ≥10 days, excluding the last recording in the three with mouth leakage, was 237 ml/day (SD = 75), significantly less than the final reading in those with mouth leakage (mean ± SD 343 ± 40 ml/day, $p < 0.05$). This fluid appeared to contain excessive mucus. Viscometry demonstrated readings that varied with shear rate (Fig. 2) and were higher than those we have previously obtained in fetal lung liquid (mean ± SD 0.75 ± 0.06 mPa), which did not vary with shear rate.

As often found in chronic fetal preparations, the body weights of chronically instrumented fetuses were reduced (by 18%) compared with those of the uninstrumented controls. However, relative to body weight, there was no significant difference between fetuses undergoing chronic pharyngeal drainage and their control cotwins in lung weight, either before or after in-

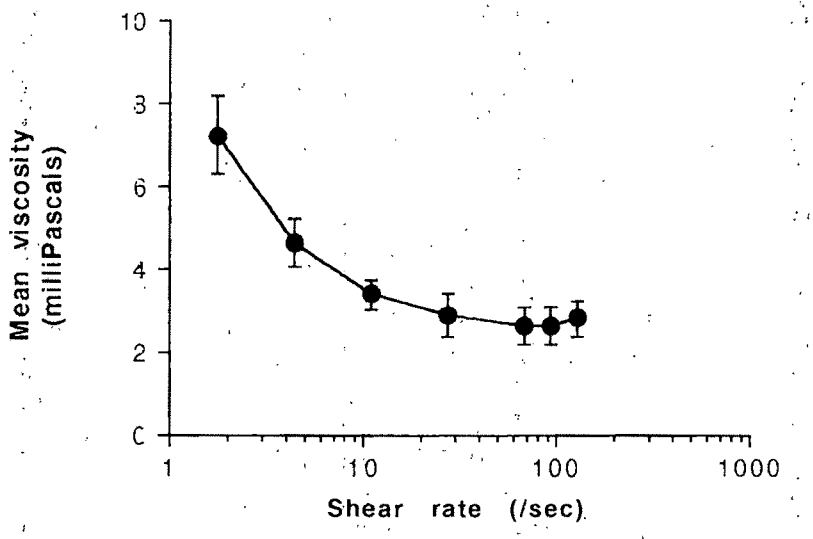


Fig. 2. Viscosity mean ± SEM in nasopharyngeal fluid collected on day 8 to 10 of drainage varies with shear rate, indicating its nonnewtonian nature.

Table I. The effect of chronic nasopharyngeal drainage on fetal lung anatomy and DNA (n = 10 unless otherwise stated)

Parameter (mean ± SEM)	*Control fetuses*	*Experimental fetuses*	*Experimental as % of control*	*Significance, p value*
Body weight (gm)	3199 ± 303	2553 ± 204	81.8 ± 5.4	0.01
Lung/body weight ratio	0.028 ± 0.001	0.027 ± 0.002	97.9 ± 7.2	NS
Left lung postinflation/body weight ratio	0.027 ± 0.001	0.027 ± 0.002	102.7 ± 7.8	NS
Right lung total DNA (mg) (n = 8)	667.7 ± 68.9	495.3 ± 57.2	78.4 ± 12.1	NS
Right lung DNA/body weight ratio (n = 8)	195.8 ± 13.8	187.5 ± 20.1	96.9 ± 10.5	NS
Lung occupied by airspaces (%) (n = 8)	65.7 ± 2.8	63.7 ± 2.5	97.5 ± 3.8	NS

The p values are shown where <0.05, although after correction for multiple comparisons values <0.008 were required for statistical significance.

NS, Not significant.

flation, or in lung DNA, as shown in Table I. In addition, lung morphometric variables were similar in control and experimental fetuses. Experimental lung/body weight ratios as a percent of control values are shown in Fig. 3 as a function of duration of drainage. When expressed as a percentage of control, there was no significant correlation between experimental lung/body weight ratio, inflated lung/body weight ratio, cross-sectional airspace area, or lung DNA/body weight ratio, with either pharyngeal-amniotic pressure or duration of drainage.

In spite of the negative pharyngeal pressure relative to amniotic pressure, tracheal pressure relative to amniotic pressure remained positive (1.7 ± 0.9 mm Hg), similar to control readings (1.5 ± 0.1 mm Hg), and significantly greater than contemporaneous measurements of pharyngeal-amniotic pressure (−3.0 ± 0.9 mm Hg, p = 0.002). Thus the increase in the tracheal-pharyngeal pressure gradient in drained fetuses (5.8 ± 1.2 mm Hg) compared with controls (0.8 ± 0.2 mm Hg, p = 0.02) was attributable to the reduction in pharyngeal pressure, rather than any alteration in tracheal pressure.

Comment

This study demonstrates that in fetal sheep mimicking the effect of low amniotic pressure on the upper airway by reducing pharyngeal pressure does not affect pulmonary development. We had previously hypothesized that a reduction in amniotic pressure of >2 mm Hg should impair fetal lung development by producing a net loss of lung fluid, because the rate of lung liquid escape would then exceed that at which lung liquid was produced.[15] Chronic fetal pharyngeal drainage in these experiments significantly reduced pharyngeal-amniotic pressure compared to controls and mimicked a drop in amniotic pressure at the laryngeal outlet of approximately 3.5 mm Hg. This was smaller than would be predicted for a column of newtonian fluid in a rigid tube in a closed system; in our in vivo experiment, however, the fetal pharynx was nonrigid, the fluid nonNewtonian (i.e., viscosity varied with shear rate)

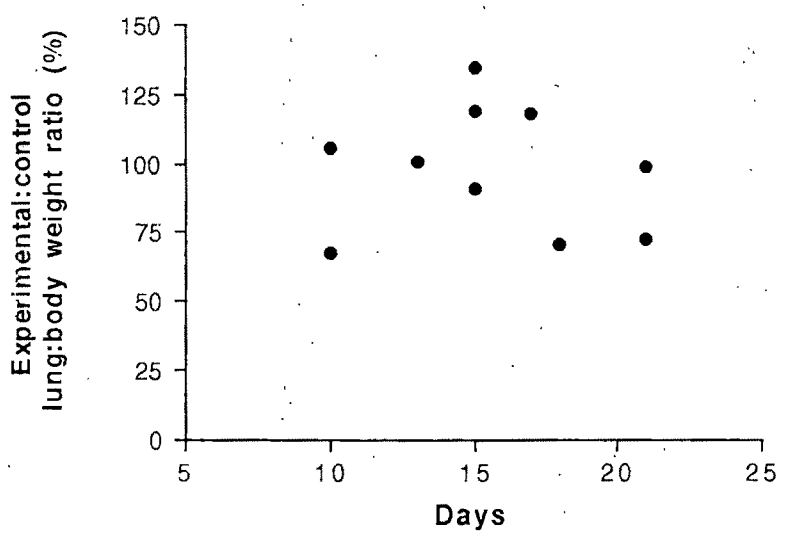


Fig. 3. Lung/body weight ratio in experimental twin as percentage of that in control twin expressed as function of duration of nasopharyngeal drainage.

and highly viscous, and the fetus continuously introducing more fluid into the system. Nevertheless, the degree of pressure reduction achieved in this study was similar to that found in human oligohydramnios[14, 15] and slightly greater than that reported in experimental oligohydramnios in the sheep.[11]

Testing our hypothesis necessitated a study design that did not produce oligohydramnios. Accordingly, we isolated the pharynx from the amniotic cavity and took care to replace the drained volume intraamniotically. A 10-day drainage period was chosen as the minimum necessary to produce detectable impairment in lung development. Fewell et al.[25] reported significantly reduced lung/body weight ratios after 10 to 22 days' phrenic denervation in six fetal lambs, three of which were subjected to this insult for ≤15 days. With the same lung processing techniques as in the current study, we recently found, by comparison with control cotwins, that 21 days' tracheal drainage reduced the lung/body weight ratio by 40%, both before and after inflation, and the lung DNA/body weight ratio and the percentage of lung occupied by airspaces by 26%.[26] None of these parameters was affected by 10 to 21 days' chronic pharyngeal drainage. Furthermore, none showed any correlation with increasing duration of drainage, which might be expected if the negative results were attributable to the minimum drainage period being too short.

The normal standing pressure in the fetal trachea is due to the resistance to egress of lung fluid provided during apnea by an active laryngeal retentive mechanism.[27-29] Bypassing this resistance in tracheostomy experiments allows free escape of fluid along the tracheal-amniotic pressure gradient, leading to equilibration of tracheal and amniotic pressures.[18, 20] The lack of effect on lung growth compared with controls in this experiment may be explained by the failure of pharyngeal drainage to alter standing tracheal pressure (which remained similar to values in our intact fetuses and those of others[7, 17, 18]). We speculate that the negative pressure generated within the fetal pharynx did not impair laryngeal retention to increase lung fluid escape; thus there was no flow-related reduction in standing tracheal pressure. The unexpectedly viscous nature of the pharyngeal fluid may have created unphysiologic conditions in the pharynx, possibly preventing any increase in lung liquid escape otherwise attributable to altered pressure gradients alone. The concentration of mucus secretions within the pharynx presumably occurred either in response to the presence of a foreign body in proximity to mucous membranes or by preventing dilution of secretions in amniotic fluid during mouth opening and swallowing.

Fewell and Johnson,[18] in contrast to other workers,[7, 17, 27-29] have claimed that the major resistance to lung liquid egress occurs not at the larynx but in the buccal cavity.[1] They found supraamniotic pressures in the buccal cavity of fetal sheep similar to those in the trachea and were able to eliminate the standing tracheal pressure with a pharyngeal tube into the amniotic cavity. The lack of effect we observed of chronic nasopharyngeal drainage at subamniotic pharyngeal pressures, both on tracheal pressure and on lung development, does not support their contention.

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Stimulation of ovarian tumor cell proliferation with monocyte products including interleukin-1, interleukin-6, and tumor necrosis factor-α

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OBJECTIVE: We investigated whether monocyte-derived factors could stimulate the growth of ovarian cancer cells.

STUDY DESIGN: Human peripheral blood monocytes or human monocyte–like cell lines THP-1 and U-937 were cultured with or without macrophage colony-stimulating factor, lipopolysaccharide, or phorbol myristate acetate. Culture supernatants or recombinant cytokines were assayed for growth stimulation of ovarian cancer cell lines by tritium-thymidine incorporation and direct cell counts followed by statistical analysis with Student *t* test.

RESULTS: Conditioned medium from peripheral blood monocytes or from THP-1 or U-937 cells stimulated ovarian cancer cell growth. Interleukin-1α, tumor necrosis factor-α, and interleukin-6 also stimulated ovarian cancer cell growth, whereas macrophage, granulocyte, and granulocyte-macrophage colony-stimulating factor did not. Concentrations of tumor necrosis factor, interleukin-1, and interleukin-6 in conditioned medium could not account for all the growth stimulation, and activity remained after neutralization of tumor necrosis factor, interleukin-1, and interleukin-6 with antibodies.

CONCLUSIONS: Interleukin-1, interleukin-6, tumor necrosis factor, and additional monocyte factor(s) could provide paracrine growth stimulation when monocytes are attracted to ovarian cancers that produce macrophage colony-stimulating factor. (Am J Obstet Gynecol 1992;166:997-1007.)

Key words: Monocyte, growth factor, ovarian cancer

Epithelial ovarian cancer is the leading cause of death from gynecologic malignancy.[1] Macrophages are associated with ovarian cancer cells within solid tumor implants and ascites fluid.[2, 3] Understanding the interaction between ovarian tumors and host macrophages may aid in developing novel strategies for therapy. Previous work has indicated that ovarian cancer cells can produce macrophage colony-stimulating factor (CSF),[4, 5] which augments monocytopoiesis and acts as a chemoattractant for monocytes.[6] Macrophage CSF can also act on mature monocytes and macrophages, stimulating increased production of tumor necrosis factor-α (TNF-α).[7]

TNF-α is cytotoxic or cytostatic for some but not all tumor cells. The growth of neoplastic B cells,[8] astrocytoma cells,[9] and human osteosarcoma cells[10] has been stimulated by TNF-α. The growth of human cervical carcinoma cell lines is inhibited by high concentrations of tumor necrosis factor but is stimulated by low concentrations.[11] Interleukin-1 (IL-1), another monocyte product, can stimulate growth of a human astrocytoma cell line[9] and malignant trophoblastic cells.[12] Interleukin-6 (IL-6), produced by both macrophages and tumor cells, can stimulate growth of a human myeloma cell line and may act as an autocrine growth factor for human renal cell carcinomas.[13, 14]

Although it is known that monocytes or monocyte products can stimulate the growth of ovarian tumor cells and cell lines,[15-17] the mechanism underlying this effect is not understood. The present study was undertaken to characterize monocyte-derived factor(s) that stimulate growth of ovarian carcinoma cells.

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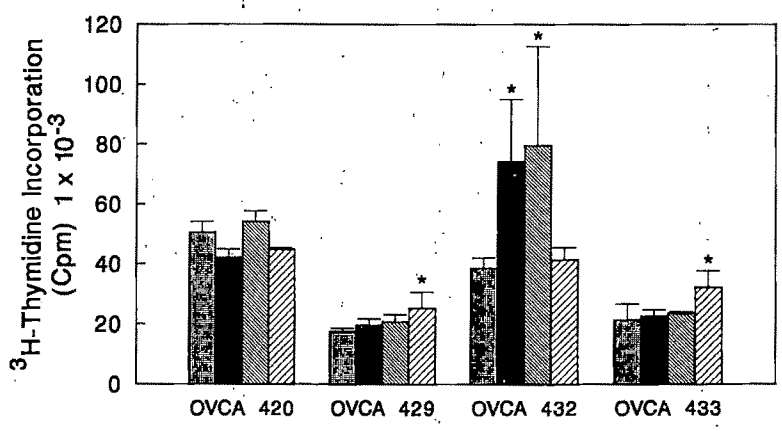


Fig. 1. ^{3}H-thymidine incorporation by ovarian carcinoma cell lines in medium conditioned by peripheral blood monocytes. Each ovarian carcinoma cell line was incubated with 0, 1%, 10% and 100% of peripheral blood monocyte–conditioned medium (*bars*, from left to right). Significant stimulation (*asterisk*) was observed with OVCA 429 ($p = 0.03$), OVCA 432 ($p = 0.01$), and OVCA 433 ($p = 0.03$).

Material and methods

Growth factors. Recombinant macrophage CSF (2×10^6 U/mg) was generously provided by Genetics Institute (Cambridge, Mass.). Recombinant granulocyte-macrophage CSF (1×10^8 U/mg) and granulocyte CSF (1×10^8 U/mg) were provided by AMGEN (Thousand Oaks, Calif.). Recombinant TNF-α (2×10^7 U/mg) was purchased from Genzyme (Boston). Recombinant IL-1α (5×10^8 U/mg) was purchased from R&D systems (Minneapolis). IL-6 (1×10^5 U/ml) was provided by Drs. T. Kishimoto and T. Hiramo (Osaka).

Chemicals. Phorbol 12-myristate-13-acetate, lipopolysaccharide from *Escherichia coli* 0127:B8, and phenyl-Sepharose CL-4B were purchased from Sigma Chemical Company (St. Louis). Tritiated thymidine was supplied by New England Nuclear (Boston).

Cell lines. Four ovarian carcinoma cell lines were used in this study. OVCA 420, OVCA 429, OVCA 432, and OVCA 433 cells were established from ascites tumor cells of patients with ovarian carcinoma.[18] The cells were grown in Eagle's minimal essential medium that was supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mmol/L sodium pyruvate, and 1% nonessential amino acid mixture. Medium was changed every 3 days and tumor cells were subcultured once a week. For subculture and experiments, monolayers were detached with 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid. The human acute monocytic leukemia cell line THP-1 and the human histiocytic lymphoma U-937 were obtained from the American Type Culture Collection (Bethesda, Md.). THP-1 and U-937 cells were grown in RPMI 1640 medium with 10% fetal bovine serum and antibiotics. In the case of THP-1, medium was supplemented with 5×10^{-5} mol/L β-mercaptoethanol.

Monocytes and monocyte-conditioned media. Human peripheral blood mononuclear cells were obtained by Ficoll-Hypaque discontinuous density-gradient centrifugation and washed three times in Hanks' balanced salt solution. Mononuclear cells were incubated for 2 hours in T150 tissue culture flasks at a density of 1×10^6 cells per milliliter in culture medium consisting of RPMI 1640 supplemented with 10% fetal bovine serum and antibiotics. The nonadherent cells were removed from flasks by washing three times with Hanks' balanced salt solution. The residual adherent population (peripheral blood monocytes) contained >90% monocytes judged by morphologic features after Giemsa staining and by the ability to phagocytize 1 μm latex particles. Adherent cells were incubated in culture medium or RPMI 1640 with 2 mmol/L L-glutamine and antibiotics but without fetal bovine serum. Peripheral blood monocytes were treated with different concentrations of M-CSF for 24, 48, and 72 hours or with 10 μg/ml lipopolysaccharide for 48 hours at 37° C in 5% carbon dioxide and 95% humidified air. The medium was collected, centrifuged at 205*g* for 10 minutes, and passed through 0.20 μm filters. Supernatants were stored at −20° C until used for experiments.

Media conditioned by monocyte-derived cell lines. THP-1 and U-937 cells were grown in the media described above with 10% fetal bovine serum. Cells were sedimented by centrifugation at 205*g* for 10 minutes at 20° C, washed three times with Hanks' balanced salt solution, and treated with 10 μg/ml lipopolysaccharide and 10 ng/ml of phorbol 12-myristate-13-acetate in culture medium or serum-free medium at a density of 5×10^5 cells/milliliter. After 2 days the supernatants

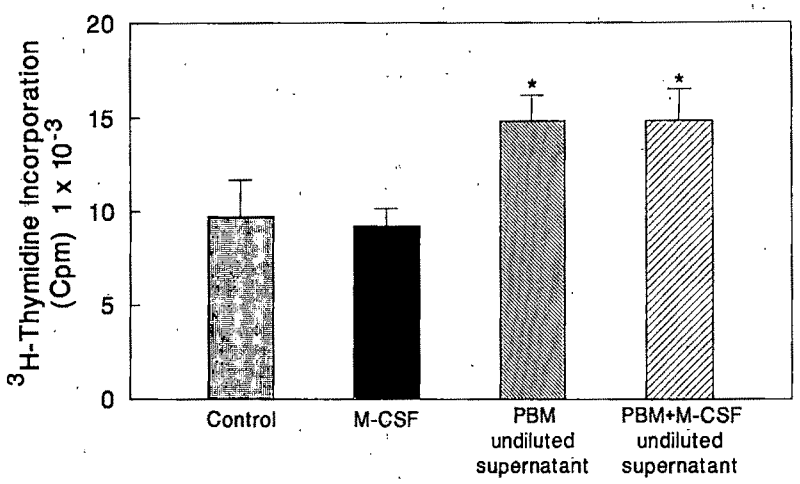


Fig. 2. Effect of macrophage CSF *(M-CSF)* on ability of peripheral blood monocytes *(PBM)* to stimulate growth of OVCA 433 cells. Peripheral blood monocyte were incubated with or without 100 ng/ml of macrophage CSF. ^{3}H-thymidine incorporation by OVCA 433 cellls was measured in presence of each undiluted conditioned medium. Macrophage CSF (100 ng/ml) was incubated with OVCA 433 as control. *Asterisk,* Significant stimulation ($p < 0.05$).

were harvested and stored under the same conditions described for monocyte-conditioned media.

^{3}H-Thymidine incorporation and cell counts. Four ovarian carcinoma cell lines were incubated at a density of 5 × 10^4 cells per 200 μl of tissue culture medium in 96-well, flat-bottomed microtiter plates. After 24 hours, the medium was removed from each well. For experiments requiring serum-free conditions, cells were washed three times with Hanks' balanced salt solution. Different concentrations of conditioned medium, cytokines, lipopolysaccharide, or phorbol 12-myristate-13-acetate diluted with tissue culture medium were added in a final volume of 200 μl. All assays were performed in quadruplicate. Cells were cultured for an additional 24 hours. Proliferation of ovarian tumor cells was measured by the addition of ^{3}H-thymidine (1 μCi per well) for the final 6 hours before harvest. After cells were labeled, culture medium was removed from each well, and cells were washed with Hanks' balanced salt solution and solubilized with 2 mol/L sodium hydroxide. Cell-associated radioactivity was measured with a Packard β-counter. For cell counts, cohort wells of ovarian carcinoma cells were incubated with growth factors and monocyte supernatant, trypsinized, and counted with a hemocytometer at 72 hours.

Biochemical characterization of the monocyte-derived growth factors. Aliquots of conditioned medium were exposed to different experimental conditions, and the residual growth-stimulating activity was measured. Heat stability was evaluated by boiling the conditioned medium for 5 minutes. Acid stability was tested by treating conditioned medium with a final concentration of 1N acetic acid (pH 2.6). Sensitivity to reduction or denaturation was assessed by subjecting aliquots of conditioned medium to a final concentration of 5% 2-mercaptoethanol, 0.1% sodium dodecyl sulfate, or 2 mol/L guanidine hydrochloride. Conditioned medium was exposed to acid or to reduction overnight at 4° C and dialyzed for 24 hours against RPMI 1640 with a dialysis membrane that retained molecules of >12 to 14 kd. The dialyzed media were diluted with culture medium and assayed for their ability to stimulate ^{3}H-thymidine incorporation by human ovarian carcinoma cell lines.

Measurement of IL-1, IL-6, and TNF-α in conditioned media. Concentrations of IL-1 were measured by bioassay with mouse thymocytes as described.[19] IL-6 was detected with enzyme-linked immunsorbent assay as described.[20] TNF-α was detected with a radioimmunoassay as described by Genzyme.

Neutralization of tumor necrosis factor, IL-1, and IL-6. Diluted conditioned medium was incubated for 2 hours at room temperature with 10^4 to 10^5 neutralizing units of polyclonal rabbit antihuman TNF-α from Genzyme or 250 to 2500 neutralizing units of goat antihuman IL-1α from R&D Systems or 10 to 100 neutralizing units of rabbit antihuman IL-6 from Genzyme. The effect on growth of ovarian tumor cells lines was measured with the ^{3}H-thymidine incorporation assay. The ability of anti–tumor necrosis factor, anti-IL-1, and anti-IL-6 to neutralize authentic tumor necrosis factor, IL-1, or IL-6 was measured in the same experiments.

Partial purification of the monocyte-derived growth factor. Ammonium sulfate was added to conditioned culture medium to provide a final concentration of 20% before separation on a phenyl-Sepharose column that had been equilibrated with 20% ammonium sulfate.

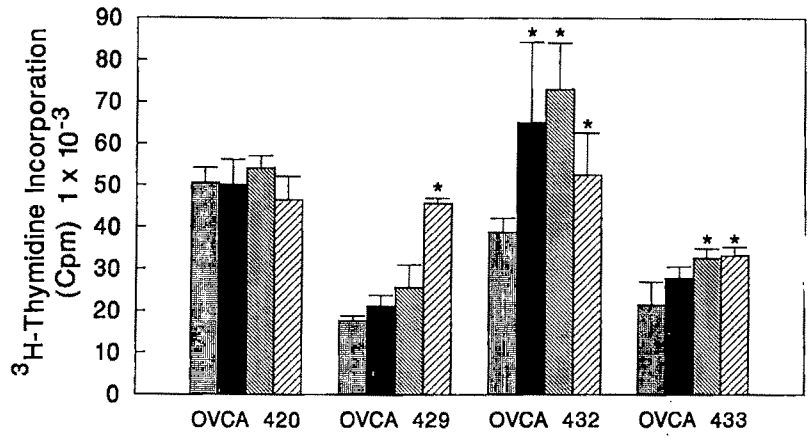


Fig. 3. ^{3}H-thymidine incorporation by human ovarian carcinoma cells in medium conditioned by peripheral blood monocytes grown in presence of lipopolysaccharide. Peripheral blood monocytes were treated with 10 μg/ml lipopolysaccharide. Each of four cell lines was grown in presence of 0%, 1%, 10%, and 100% conditioned medium, indicated by *bars* from left to right. *Asterisk,* Significant stimulation ($p < 0.05$).

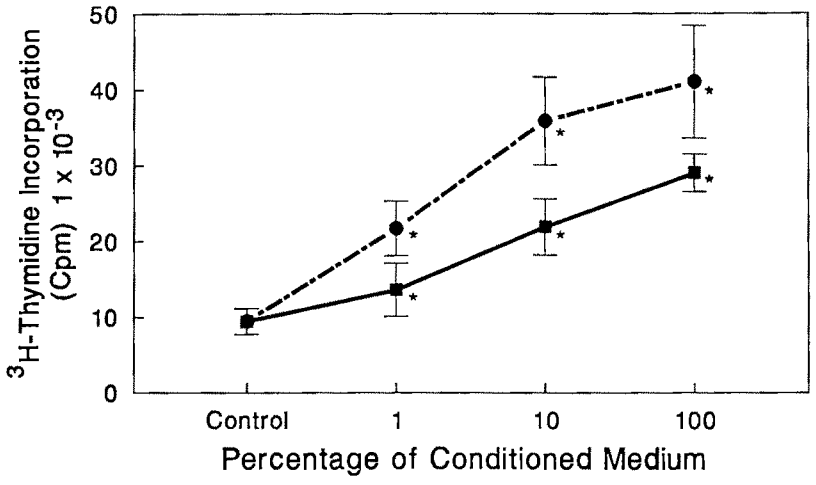


Fig. 4. ^{3}H-thymidine incorporation by OVCA 433 cells in serum-free medium conditioned by peripheral blood monocytes in the presence *(dashed line)* and absence *(solid line)* of 10 μg/ml lipopolysaccharide. *Asterisk,* significant stimulation ($p < 0.05$).

After addition of the sample, the column was washed with 50 ml of 20% ammonium sulfate in phosphate-buffered saline solution and 50 ml of deionized water. Subsequently, material was eluted with 50 ml of 5% and 50 ml of 25% ethylene glycol (Sigma). Aliquots of the eluate were dialyzed in phosphate-buffered saline solution for 24 hours at 4° C, diluted with tissue culture medium, and tested for their ability to stimulate proliferation of ovarian cancer cell lines.

Analysis. The Student *t* test was used to compare control and experimental groups. Data were expressed as mean ± SD. All assays were performed with at least four replicates.

Results

Growth stimulation of ovarian carcinoma cell lines by peripheral blood monocyte–conditioned media. Initial experiments were carried out to assess the effect of peripheral blood monocyte–conditioned medium on the growth of four ovarian carcinoma cell lines, including OVCA 420, OVCA 429, OVCA 432, and OVCA 433. The growth of OVCA 429, OVCA 432, and OVCA 433 was stimulated by peripheral blood monocyte–conditioned medium (Fig. 1). The growth-stimulating activity of the peripheral blood monocyte supernatant was not enhanced by treatment of peripheral blood monocytes for 1 to 3 days with macrophage CSF (Fig. 2). Direct addition of from 1 to 1000 ng/ml macrophage CSF to ovarian cancer cell lines did not affect growth of the ovarian cancer cells and did not enhance the stimulatory effect of peripheral blood monocyte supernatants. Neither granulocyte-macrophage nor granulocyte CSF stimulated or inhibited the growth of the four ovarian carcinoma cell lines tested (data not shown).

Enhancement of the growth-stimulating activity of

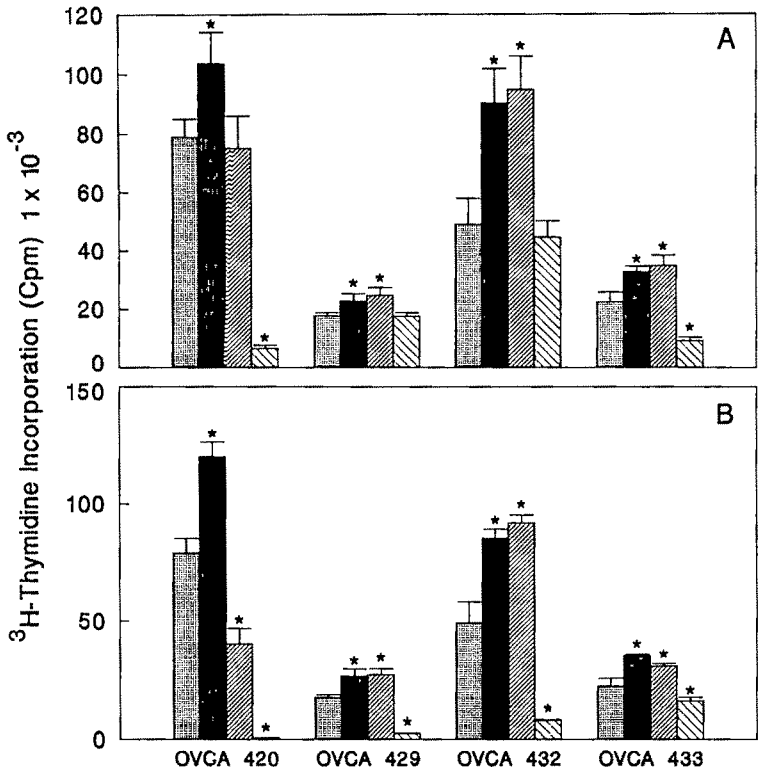


Fig. 5. A, ^{3}H-thymidine incorporation by ovarian carcinoma cell lines in medium conditioned by THP-1 cells grown in presence of 10 μg/ml lipopolysaccharide and 10 ng/ml phorbol 12-myristate-13-acetate. Each of four cell lines was grown in presence of 0%, 1%, 10%, or 100% conditioned medium, indicated by *bars* from left to right. *Asterisk*, Significant stimulation or inhibition ($p < 0.05$). **B,** ^{3}H-thymidine incorporation by ovarian carcinoma cell lines with growth medium conditioned by U-937 in presence of 10 μg/ml lipopolysaccharide and 10 ng/ml phorbol 12-myristate-13-acetate. All four cell lines could be stimulated with 1% or 10% conditioned medium, indicated by *bars* from left to right.

peripheral blood monocytes by treatment with lipopolysaccharide. When peripheral blood monocytes were treated with 10 μg/ml lipopolysaccharide, supernatants once again enhanced growth in three of four ovarian carcinoma cell lines by 55% to 159% (Fig. 3). When tested in serum-free medium, greater stimulation was noted in the presence of lipopolysaccharide than in its absence (Fig. 4). Incubation of ovarian carcinoma cell lines with 10 μg/ml lipopolysaccharide produced slight enhancement of ^{3}H-thymidine incorporation by the OVCA 420 (10%), OVCA 432 (25%), and OVCA 433 (19%) cell lines but not by OVCA 429 (data not shown).

Growth stimulation of ovarian carcinoma cell lines with media conditioned by THP-1 or U-937 in the presence of lipopolysaccharide and phorbol 12-myristate-13-acetate. The THP-1 and U-937 cell lines were established from human neoplasms of monocytic lineage. To enhance production of ovarian cancer growth-stimulating activity, both cell lines were treated with phorbol 12-myristate-13-acetate and lipopolysaccharide. The growth of all four ovarian carcinoma cell lines was significantly stimulated by low concentrations of THP-1 and U-937 products (Fig. 5, *A* and *B*). High concentrations of conditioned medium could, however, inhibit the growth of all four ovarian carcinoma cell lines (Fig. 5, *A* and *B*). In serum-free medium the growth of the four cell lines was stimulated and supernatants were active at a higher dilution (data not shown). Since phorbol 12-myristate-13-acetate and lipopolysaccharide had been added to the conditioned medium, the effect of these agents on ovarian tumor cell growth was determined in the same experiment. High concentrations of phorbol 12-myristate-13-acetate (10 ng/ml) in combination with lipopolysaccharide (10 μg/ml) could stimulate growth of OVCA 429, OVCA 432, and OVCA 433 but inhibited growth of OVCA 420 (Fig. 6). Lower concentrations of 0.1 to 1 μg/ml lipopolysaccharide in combination with 0.1 to 1 ng/ml phorbol 12-myristate-13-acetate failed to affect tumor cell growth.

The effect of TNF-α, IL-1, and IL-6 on growth of ovarian carcinoma cell lines. TNF-α and IL-1 stimulated the growth of OVCA 429, OVCA 432, and OVCA 433 (Fig. 7, *A* and *B*). To stimulate growth of OVCA 433 required >100 U/ml for TNF-α and 50 U/ml for IL-1. OVCA 429 and OVCA 432 were much more sensitive than OVCA 433 to TNF-α and IL-1. No greater

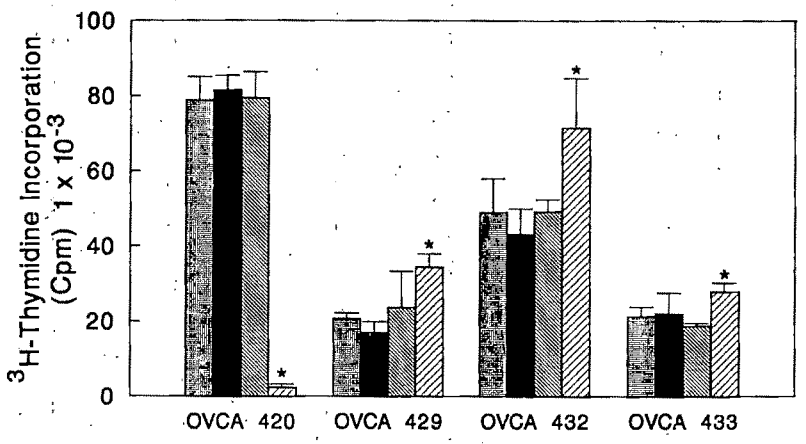


Fig. 6. Effect of phorbol 12-myristate-13-acetate and lipopolysaccharide on ^{3}H-thymidine incorporation by ovarian carcinoma cell lines. Each of four cell lines was incubated with diluent, 0.1 μg/ml lipopolysaccharide plus 0.1 ng/ml phorbol 12-myristate-13-acetate, 1 μg/ml lipopolysaccharide plus 1 ng/ml phorbol 12-myristate-13-acetate, or 10 μg/ml lipopolysaccharide plus 10 ng/ml phorbol 12-myristate-13-acetate, indicated by *bars* from left to right. Three of four cell lines, OVCA 429 ($p = 0.0004$), OVCA 432 ($p = 0.03$), and OVCA 433 ($p = 0.009$), were significantly stimulated, but OVCA 420 ($p = 0.0000003$) was inhibited by 10 μg/ml lipopolysaccharide plus 10 ng/ml phorbol 12-myristate-13-acetate.

stimulation was found at higher concentrations of TNF-α (up to 10^4 U/ml) or IL-1 (50 to 5000 U/ml). All four cell lines were slightly stimulated by 1 U/ml of IL-6 (Fig. 7, *C*). When ovarian cancer cell lines were treated with all three growth factors in combination, no additive or superadditive effects were observed (data not shown). No growth inhibition was observed after 24 hours incubation with any concentration of TNF-α, IL-1, or IL-6.

Growth of ovarian tumor cell lines was studied in the presence of 10% fetal bovine serum or under serum-free conditions. Three of the four cell lines could be stimulated with TNF-α, IL-1 or peripheral blood monocytes in either medium. Under serum-free conditions, lower concentrations of the factors were required. In the presence of serum, growth of the fourth cell line, OVCA 420, was not affected by TNF-α, IL-1, or peripheral blood monocyte–conditioned medium. In serum-free medium proliferation of OVCA 420 could be stimulated by IL-1 (Fig. 8), TNF-α, and peripheral blood monocyte–conditioned medium (data not shown). Stimulation of the growth of ovarian tumor cells was also confirmed by direct cell counts (Table I).

Concentrations of TNF-α, IL-1, and IL-6 in medium conditioned by peripheral blood monocyte and monocyte-derived cell lines. Relatively low concentrations of IL-1 (4.3 to 27.3 U/ml) and TNF-α (10 to 22 U/ml) were detected in all peripheral blood monocyte–conditioned medium (Table II). In the absence of lipopolysaccharide, IL-6 was not detected in supernatants from peripheral blood monocyte–conditioned medium (Table II). Peripheral blood monocyte–conditioned medium from three of the four donors significantly stimulated growth of three ovarian cancer cell lines. Peripheral blood monocytes from donor A in Table II, for example, stimulated growth of OVCA 429, OVCA 432, and OVCA 433 in Fig. 1. In the presence of lipopolysaccharide, peripheral blood monocytes produced significant amounts of IL-6, achieving concentrations of 19.5 to 27 U/ml (Table II). Low concentrations of IL-6 (1 U/ml) could stimulate each of the four cell lines (Fig. 7, *C*). Supernatants from peripheral blood monocytes and from lipopolysaccharide-treated THP-1 and U-937 cells contained detectable quantities of TNF-α (6 and 90 U/ml), IL-1 (1003 and 1294 U/ml), and IL-6 (0.7 and 9.5 U/ml). Antisera to TNF-α, IL-1, or IL-6 could neutralize authentic tumor necrosis factor, IL-1, and IL-6 but failed to neutralize the growth-stimulating activity of lipopolysaccharide-treated peripheral blood monocytes (Table III) or THP-1–conditioned medium (data not shown).

Characterization of stimulatory factor(s) from peripheral blood monocytes and THP-1. Several approaches were taken to characterize the factor(s) in conditioned media that were responsible for stimulating growth of ovarian carcinoma cell lines. Stimulatory activity in the supernatants of peripheral blood monocytes and THP-1 cells were destroyed by boiling for 5 minutes but remained intact after incubation overnight at pH 2.6 or in the presence of reducing and denaturing agents (Table IV). Preservation of activity after reduction and dialysis suggests that factors of >12 to 14

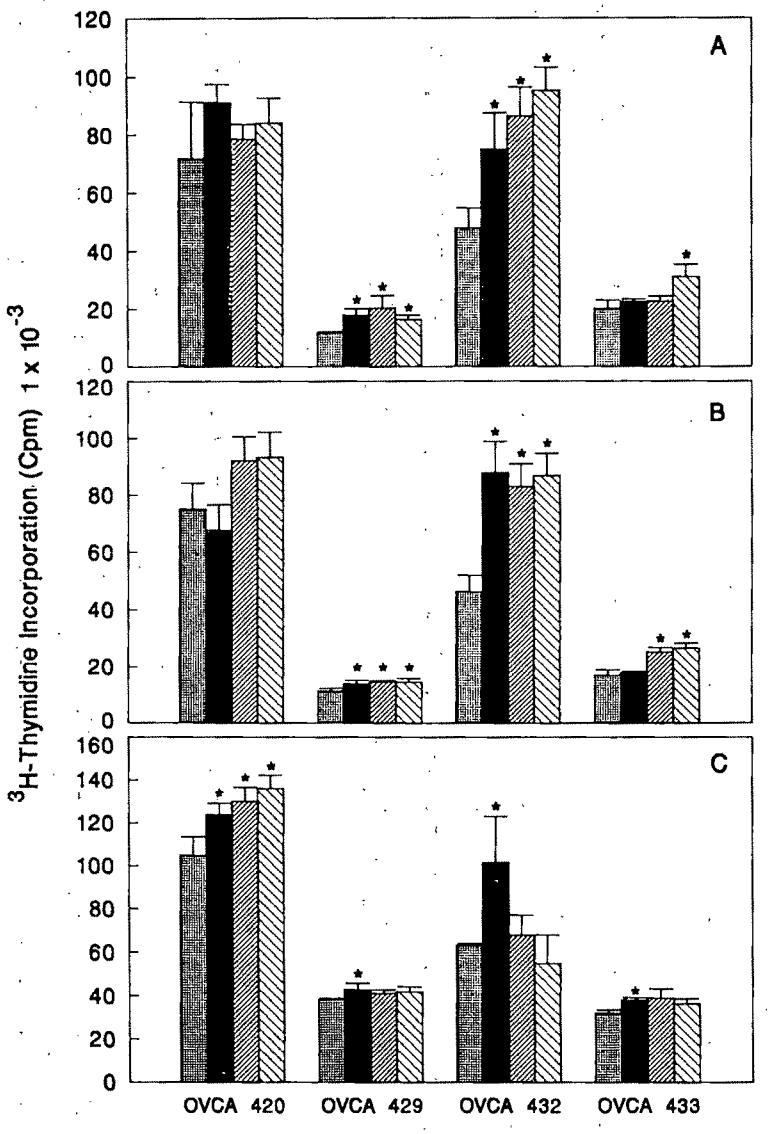


Fig. 7. A, Effect of TNF-α on ^{3}H-thymidine incorporation by ovarian carcinoma cell lines. Ovarian cancer cell lines were incubated with 0, 10, 100, and 1000 U/ml TNF-α, indicated by *bars* from left to right. *Asterisk,* Significant stimulation ($p < 0.05$). **B,** Effect of IL-1 on ^{3}H-thymidine incorporation by ovarian carcinoma cell lines. Ovarian carcinoma cells were incubated with 0, 5, 50, and 500 U/ml IL-1, indicated by *bars* from left to right. *Asterisk,* Significant stimulation ($p < 0.05$). **C,** Effect of IL-6 on ^{3}H-thymidine incorporation by ovarian carcinoma cell lines. Ovarian cancer cells were incubated with 0, 1, 10, and 100 U/ml IL-6, indicated by *bars* from left to right. *Asterisk,* Significant stimulation ($p < 0.05$).

kd are involved or that low-molecular-weight factors are tightly bound to larger moieties.

Partial purification of growth-stimulatory factors. The growth-stimulatory activity from peripheral blood monocyte and THP-1 cells could be recovered from a phenyl-Sepharose column. After washing with 5% ethylene glycol, most growth-stimulatory activity was obtained in the first 15 ml fraction. After washing with an additional 50 ml of 25% ethylene glycol, a second peak of activity was recovered (data not shown). Activity recovered from phenyl-Sepharose was retained after concentration on a YM10 (Amicon) filter and subsequent dialysis. Growth-stimulatory activity from either peripheral blood monocytes or THP-1 had similar characteristics on partial purification.

Comment

Our previous work indicated that ovarian carcinoma cell lines express macrophage CSF.[5] In our current study exogenous macrophage CSF failed to exert a di-

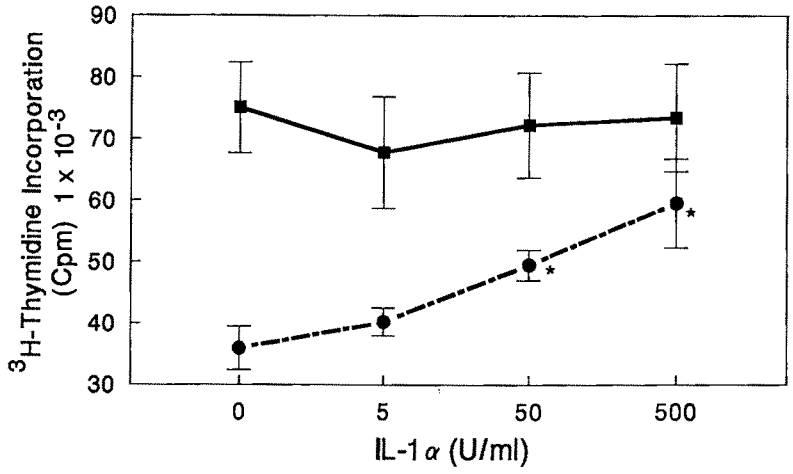


Fig. 8. Effect of IL-1 on ^{3}H-thymidine incorporation by OVCA 420 cells in serum-free medium *(dashed line)* or in presence of 10% fetal bovine serum *(solid line)*. *Asterisk*, Significant stimulation ($p < 0.05$).

Table I. Comparison of ^{3}H-thymidine incorporation and cell number of ovarian cancer treated with IL-1, TNF-α, IL-6, and monocyte (THP-1) supernatant

	OVCA 420		*OVCA 429*		*OVCA 432*		*OVCA 433*	
Conditions	*Counts/min*	*Cells × 10^4*	*Counts/min*	*Cells × 10^4*	*Counts/min*	*Cells × 10^4*	*Counts/min*	*Cells × 10^4*
Medium	126,744	4.5	9,954	3.0	217,631	5.5	15,926	2.5
IL-1 (500 U/ml)	151,695*	10.0	33,448*	5.0	264,601*	10.0	17,353	3.5
IL-1 (50 U/ml)	149,521*	8.5	28,329*	3.5	256,781*	5.0	14,728	2.0
TNF (1000 U/ml)	167,573*	5.5	64,055*	7.5	259,567*	10.0	39,501*	4.0
TNF (100 U/ml)	224,610*	8.5	75,759*	9.5	264,913*	6.5	35,609*	5.5
THP-1 (100%)	195,136*	8.5	80,421*	9.5	252,696*	7.5	36,353*	6.0
THP-1 (10%)	157,797*	7.5	31,379*	6.0	270,960*	6.5	17,178	2.4

Ovarian cancer cells (2×10^4) were incubated in quadruplicate with above growth factors in serum-free medium, and ^{3}H-thymidine incorporation was performed at 24 hours (counts per minute, mean ± SD). SD was <15% for all conditions. Cell counts were performed at 72 hours in cohort wells (mean number of cells per well).

*Significant stimulation ($p < 0.05$).

rect effect on growth of these same cell lines. Proliferation may have been maximally stimulated by endogenous macrophage CSF. As these particular cell lines do not, however, express *fms* and do not bind macrophage CSF, it seems more likely that they lack receptors for the growth factor.

Macrophage CSF is known, however, to stimulate monocytopoiesis and to be a potent chemoattractant for monocytes. Consequently, we have examined the effect of monocyte products on growth of ovarian carcinoma cell lines. Monocytes, macrophages, and their products have previously been shown to stimulate clonogenic growth of ovarian cancer.[15-17] In this study we found that peripheral blood monocytes produced factors that stimulated the proliferation of ovarian carcinoma cell lines judged by ^{3}H-thymidine incorporation. Different ovarian cancer cell lines exhibited different responses to conditioned media from peripheral blood monocytes or monocyte-derived cell lines. OVCA 432 cells were markedly stimulated by conditioned media, even after dilution. Other ovarian cancer cell lines were less sensitive to the growth stimulatory properties of monocyte-derived factors. Lipopolysaccharide increased the production of growth-stimulatory activity by peripheral blood monocytes, producing culture supernatants with higher titer. Lipopolysaccharide alone had little effect on proliferation of the ovarian cancer cell lines, suggesting that the increased activity resulted from the effect of lipopolysaccharide on the monocytes.

The response of ovarian carcinoma cells to the stimulatory activity of monocyte-derived products is greatly influenced by the concentration of serum in the culture medium. Growth of OVCA 429, OVCA 432, and OVCA 433 was significantly enhanced by peripheral blood monocyte–conditioned medium in the presence or absence of serum. OVCA 420 cells were not sensitive to

Table II. Concentrations of TNF-α, IL-1, and IL-6 in monocyte-conditioned medium

Conditioned medium	TNF-α		IL-1	IL-6	
	ng/ml	U/ml	(U/ml)	ng/ml	U/ml
Peripheral blood monocytes (A)	0.9	18	4.3	<0.1	<0.5
Peripheral blood monocytes plus lipopolysaccharide (A)	3.7	74	60.2	3.9	19.5
Peripheral blood monocytes (B)	0.5	10	18.4	<0.1	<0.5
Peripheral blood monocytes plus lipopolysaccharide (B)	.9	18	29.3	—	
Peripheral blood monocytes (C)	1.1	22	27.3	<0.1	<0.5
Peripheral blood monocytes plus lipopolysaccharide (C)	0.7	14	40.9	—	
Peripheral blood monocytes (D)	—		—	<0.1	<0.5
Peripheral blood monocytes plus lipopolysaccharide (D)	—		—	5.4	27
THP-1 plus phorbol 12-myristate-13-acetate plus lipopolysaccharide	0.3	6	1003	0.1	0.5
U-937 plus phorbol 12-myristate-13-acetate plus lipopolysaccharide	4.5	90	1294	1.9	9.5

Conditioned medium was obtained with peripheral blood monocytes from four donors (*A* to *D*). Assays were performed as described in Material and methods. A total of 1 ng of TNF-α = 20 U; 1 ng of IL-6 = 5 U.

Table III. Neutralization of TNF-α, IL-1, or IL-6 in peripheral blood monocyte–conditioned medium with anti-TNF-α, anti-IL-1, and anti-IL-6

Antibodies	^{3}H-thymidine incorporation by OVCA 433 cells (counts/min)					
	Diluent	Tumor necrosis factor (1000 U/ml)	IL-1 (500 /ml)	IL-6 (5 U/ml)	Tumor necrosis factor plus IL-1 plus IL-6	Peripheral blood monocyte–conditioned medium (10%)
Control	33,782 ± 469	44,333 ± 1,576*	47,600 ± 2,097*	38,691 ± 1,782*	41,971 ± 1,004*	43,752 ± 2,661*
Anti-tumor necrosis factor		35,935 ± 3,329				50,570 ± 1,933*
Anti-IL-1			32,655 ± 1,020			47,068 ± 1,094*
Anti-IL-6				30,039 ± 2,720		43,715 ± 3,743*
Anti-tumor necrosis factor plus anti-IL-1 plus anti-IL-6					34,743 ± 1,883	44,601 ± 2,557*

Supernatants from lipopolysaccharide-treated peripheral blood monocytes were diluted 1 : 10 and incubated with 10^5 neutralizing units of anti-TNF-α, 2500 neutralizing units of anti-IL-1, 100 neutralizing units of anti-IL-6, or the three antibodies together. Similar incubations were performed with solutions of known growth factors. Supernatants were assayed for the ability to stimulate ^{3}H-thymidine incorporation into OVCA 433 cells. For tumor necrosis factor plus IL-1 plus IL-6, tumor necrosis factor = 1000 U/ml, IL-1 = 500 U/ml, IL-6 = 5 U/ml.

*Significant stimulation ($p < 0.002$).

peripheral blood monocyte–conditioned medium in the presence of 10% fetal bovine serum but could be stimulated significantly under serum-free conditions, suggesting that the peripheral blood monocyte–derived growth factor(s) could replace fetal bovine serum growth factor(s). Similar observations have been reported by Kirstein and Baglioni[10] who demonstrated that treatment with tumor necrosis factor can stimulate the growth of an osteosarcoma cell line in culture medium that contained 0.5% serum but that tumor necrosis factor was less active in the presence of 10% fetal bovine serum.

The growth-stimulating activity of TNF-α, IL-1, and IL-6 could not account for all of the proliferation observed when ovarian cancer cells were grown in medium conditioned by peripheral blood monocytes or monocyte-derived cell lines. Relatively low concentrations of tumor necrosis factor and IL-1 were detected in peripheral blood monocyte–conditioned medium. As relatively high concentrations of factors were required to stimulate growth of OVCA 433 cells, these factors alone cannot account for the magnitude of the stimulation observed. IL-6 could not be detected in medium conditioned by peripheral blood monocytes in the absence of lipopolysaccharide, yet this medium could stimulate ovarian tumor growth. Moreover,

Table IV. Characteristics of growth-stimulating activity in peripheral blood monocyte– and THP-1–conditioned medium

Treatment	*Stimulation of OVCA 433 cell growth*	
	Peripheral blood monocytes	*THP-1*
Control	+	+
Boiling 5 min	–	–
1N Acetic acid	+	+
5% β-Mercaptoethanol	+	+
0.1% Sodium dodecyl sulfate	+	+
2 mol/L Guanidine hydrochloride	+	+
Retained during dialysis	+	+

Peripheral blood monocyte– and THP-1–conditioned medium were treated as described in Material and methods. The treated supernatants were tested for their ability to stimulate ^{3}H-thymidine uptake by OVCA 433. *Plus sign,* Statistically significant stimulation of tumor growth.

growth-stimulatory activity could not be removed completely after neutralization of tumor necrosis factor, IL-1, and IL-6 with antisera that removed the activity of the authentic growth factors.

In addition to TNF-α, IL-1, and IL-6, monocytes also produce macrophage, granulocyte-macrophage, and granulocyte CSF. In our study macrophage, granulocyte-macrophage, and granulocyte CSF failed to stimulate growth of ovarian cancer cell lines. Our data are consistent with the possibility that exogenous hematopoietic factors could be administered during chemotherapy without stimulating tumor growth.

Monocyte-derived cell lines can be used as sources to purify immunologically active mediators.[21] In our study THP-1 and U-937 both produced ovarian tumor growth factor(s) similar to those produced by human peripheral blood monocytes. Treatment of THP-1 and U-937 with phorbol 12-myristate-13-acetate and lipopolysaccharide enhanced the production of factor(s) that stimulated all four ovarian tumor cell lines. Growth-stimulatory activity of THP-1 and U-937 was not due solely to the presence of phorbol 12-myristate-13-acetate and lipopolysaccharide. Phorbol 12-myristate-13-acetate alone or in combination with lipopolysaccharide stimulated the growth of OVCA 429, OVCA 432, and OVCA 433 cell lines but greatly inhibited the growth of OVCA 420. Moreover, THP-1 and U-937 supernatants were 10 to 100 times more potent than equivalent concentrations of phorbol 12-myristate-13-acetate and lipopolysaccharide (Figs. 5 and 6). The stimulatory activity of THP-1 and U-937 supernatants is abolished by heating, whereas phorbol 12-myristate-13-acetate and lipopolysaccharide are heat stable. Retention of activity within a dialysis membrane also argues against a significant role for phorbol 12-myristate-13-acetate in producing the stimulatory activity observed in supernatants from monocyte-derived cell lines. Observations in Table IV indicate that the THP-1–derived growth factor is very similar to the peripheral blood monocyte–derived ovarian tumor cell growth factor.

Thus our results indicate that macrophage-derived factor(s) can stimulate proliferation of ovarian tumor cells. Tumor necrosis factor, IL-1 and IL-6 may contribute to this growth stimulation, but additional factors are probably important. Further definition of the growth stimulating factor(s) produced by monocytes could permit development of new therapeutic strategies for this disease.

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Effects of chronic maternal anemia on systemic and uteroplacental oxygenation in near-term pregnant sheep

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OBJECTIVE: The hypothesis of our study was that both the systemic and uteroplacental circulations would adapt to chronic maternal anemia to ensure that oxygen supply to maternal tissues would be adequate.

STUDY DESIGN: We measured cardiac output and uteroplacental blood flow and calculated systemic and uteroplacental oxygen delivery, extraction, and consumption in pregnant sheep that were anemic for 6 days (hematocrit 14%) and in normal sheep (hematocrit 28%).

RESULTS: When compared with normal pregnant sheep, anemic pregnant sheep had increases in cardiac output and uteroplacental blood flow, neither of which was sufficient to prevent systemic or uteroplacental oxygen delivery from decreasing. In spite of decreases in oxygen delivery, systemic and uteroplacental oxygen consumptions were maintained at normal levels because of increases in oxygen extraction.

CONCLUSION: Maternal systemic and uteroplacental circulations are capable of adapting well to chronic maternal anemia. (AM J OBSTET GYNECOL 1992;166:1007-12.)

Key words: Cardiac output, uteroplacental blood flow, oxygen consumption, oxygen extraction

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During pregnancy the mother and fetus share the maternal oxygen supply. In situations where maternal oxygen supply is limited, both mother and fetus could be affected adversely. Maternal anemia leads to a decrease in blood oxygen concentration and may therefore reduce oxygen supply to maternal or fetal tissues. There are two basic compensatory responses that tis-

sues can make if oxygen concentration and thus oxygen supply are decreased.[1] The first response is an increase in blood flow, which serves to maintain a high capillary P_{O_2} and thus enhances the P_{O_2} gradient between capillary blood and tissues. The second adaptation is an increase in oxygen extraction, which results mainly from increases in capillary density. Increases in capillary density serve to reduce the distances across which oxygen must diffuse from capillaries to tissues. With either adaptation, oxygen transport to tissues would be enhanced.

The effects of acute anemia on maternal and uteroplacental oxygenation have been studied. In near-term sheep, uteroplacental blood flow does not change over a wide range of maternal hematocrits[2]; the principal compensatory response to acute anemia is an increase in oxygen extraction by uteroplacental tissues. In contrast, in the systemic circulation cardiac output increases during mild degrees of anemia, but with severe anemia (hematocrit $\leq$ 15%) cardiac output returns to values observed in nonanemic sheep.[3] Although these studies[2, 3] shed some light on the adaptation to anemia during pregnancy, it is quite likely that the responses to acute anemia would differ from those seen with anemias of longer duration. The most obvious reason for such a difference in response would be that some adaptative processes require time to develop. This is particularly likely in the case of the uteroplacental vascular bed, in which blood flow increases profoundly during normal pregnancy.[4] Long-term anemia might influence the development of the microcirculation to allow perfusion to increase even further. Although the uteroplacental adaptation to chronic anemia is of particular importance to fetal well-being, this regional response is only one portion of the total maternal circulatory response to anemia. It is not known whether the uteroplacental adaptation is typical or atypical of the mother's general response to anemia.

We designed the following series of studies to address two questions: (1) Does the uteroplacental response to chronic anemia differ substantially from the maternal systemic response? (2) Do the uteroplacental and systemic responses to chronic anemia during pregnancy differ from those observed with acute anemia?

Methods

We performed surgery on 11 pregnant sheep with singleton fetuses with a mean gestational age of 117 ± 3 days. The procedures to be described were reviewed and approved by the Institutional Animal Care and Use Committee of Magee-Womens Hospital. After inhalation anesthesia was induced with halothane, we cannulated a maternal hind limb artery and a vein.[2, 3] These catheters were advanced into the descending aorta and inferior vena cava. We catheterized the right and left ventricles via an external jugular vein and a carotid artery.[3] The maternal abdomen was then opened, a catheter was placed into a main vein of the uterine horn containing the fetus, and the catheter was advanced approximately 3 cm distal to the ovary.[2] All incisions were closed. Catheters were brought out subcutaneously to the sheep's flank where they were stored in a pouch.

We allowed all animals to recover for a mean of 2 days before any experiments were performed. While each animal stood unrestrained in a transport cage, we measured maternal blood pressure and heart rate continuously with Statham P23db transducers and recorded this information on a Beckman R611A polygraph recorder. We then measured systemic and uteroplacental blood flows and oxygen deliveries, extractions, and consumptions as baseline measurements in all 11 animals. To make these measurements we obtained blood samples from the descending aorta, uterine vein, and right ventricle. These samples were analyzed for hemoglobin concentration, hematocrit, blood gases and pH, and blood oxygen saturation, with standard techniques.[2, 3] We then injected 2 to 5 × 10^6 radionuclide-labeled microspheres, labeled with iodine 125, cerium 141, chromium 51, strontium 85, niobium 95, or scandium 46 (15 μm diameter) into the left ventricle while withdrawing a reference blood sample from the descending aorta at 7.9 ml/min.[5, 6]

After these baseline measurements were made, we divided the animals into two series, a control series (n = 5) and an anemia series (n = 6). In the anemia series we slowly reduced maternal hematocrit by performing isovolemic exchange transfusions with fresh sheep plasma.[3] We exchanged 500 to 1500 ml daily until the desired maternal hematocrit of about 14% was reached. We chose this hematocrit because our studies on acute maternal anemia[2] indicated that both mother and fetus could safely tolerate such degrees of hemodilution. This goal generally required 2 or 3 days of hemodilution. The amount of exchange transfusion each day was tolerated well by the pregnant sheep. The initial hematocrit and the erythrogenic response of each sheep determined the volume of exchange used eacy day and the number of days required to reduce the hematocrit to the desired level. Thereafter, the anemia was maintained by exchange transfusion either each day or every other day for 6 days. In the control group several animals underwent sham exchange transfusions. Fetal viability was documented periodically during the study period by ultrasonographic visualization of fetal heart activity. At the end of the 6-day experimental period we repeated the measurements of maternal systemic and uteroplacental blood flows and oxygen deliveries, extractions, and consumptions as described above.[2, 3]

Table I. Effects of 6 days of anemia on some maternal hematologic variables

	Control		*Anemia*	
	Baseline	*After 6-day study period*	*Baseline*	*After 6-day study period*
Gestational age (days)	118 ± 2	126 ± 2	118 ± 3	126 ± 3
Body weight (kg)	59 ± 7	59 ± 7	60 ± 7	60 ± 7
Hematocrit (%)	28 ± 2	28 ± 4	29 ± 3	14 ± 1*
Hemoglobin concentration (gm/dl)	9.7 ± 0.5	8.9 ± 1.3	9.5 ± 1.2	4.6 ± 0.5*
Arterial blood oxygen concentration (ml/dl)	12.2 ± 0.8	11.2 ± 1.5	12.2 ± 1.5	5.8 ± 0.9*

For control studies $n = 5$; for anemia studies $n = 6$. Values are means ± SD.

*Significant difference after 6-day study period (anemia) versus baseline measurements. Note that the baseline data of the control series did not differ significantly from any of the baseline data of the anemia series.

When the experiments were completed, each sheep was killed with an overdose of sodium pentobarbital. All maternal organs were dissected, during which time catheter placements were verified visually. The uterus, placental cotyledons, placental membranes, and fetus were removed and weighed; all tissues were then incinerated at 280° C for 48 hours. The resulting tissue ash was packed into counting vials, and the amounts of each radionuclide in each organ and in the reference blood samples were measured with a γ-scintillation counter (model 1185R, Tracor) and a 1024 channel pulse-height analyzer (Ultima II, Norland). Nuclide isotope separation was performed by computer, which used a least-squares fitting analysis to determine the amount of each isotope in each sample (Nova 3, Data General; average error in calculation of corrected radioactive counts per minute of each isotope = 5%).[2, 3, 5]

We calculated cardiac output as follows:

$$\text{Cardiac output} = (\text{cpm}_{\text{total}}/\text{cpm}_{\text{ref}}) \cdot Q_{\text{ref}}$$

where $\text{cpm}_{\text{total}}$ is the total radioactive counts per minute injected in the left ventricle,[3] cpm_{ref} is the radioactive counts per minute in the reference blood sample, and Q_{ref} is the reference withdrawal rate from the maternal descending aorta.

We computed uteroplacental blood flow as follows:

$$\text{Uteroplacental blood flow} = (\text{cpm}_{\text{up}}/\text{cpm}_{\text{ref}}) \cdot Q_{\text{ref}}$$

where cpm_{up} is the radioactive counts per minute in the uterus plus placenta. The remaining abbreviations are the same as those previously given.

We calculated systemic and uteroplacental vascular resistances as the mean arterial blood pressure divided by the cardiac output or the uteroplacental blood flow, as appropriate. These calculations represent the total resistance (i.e., the sum of changes in resistance caused by reduced viscosity plus those caused by active vasoregulation) across either the systemic or uteroplacental circulation.

We calculated blood oxygen concentration (Hemoglobin concentation × 1.34 × Blood oxygen saturation), and then used the Fick principle to calculate systemic and uteroplacental oxygen deliveries, extractions, and consumption, as follows[2, 3]:

$$\text{Oxygen deliveries} = \text{blood flow } (CaO_2)$$

$$\text{Oxygen consumption} = \text{blood flow } (CaO_2 - C_vO_2)$$

$$\text{Oxygen extraction} = (CaO_2 - C_vO_2)/CaO_2, \text{ or oxygen consumption/oxygen delivery}$$

where blood flow denotes cardiac output or uteroplacental blood flow, CaO_2 is the arterial blood oxygen concentration, and C_vO_2 is the oxygen concentration in mixed venous blood (right ventricular or uterine venous blood, as appropriate).

An analysis of variance was used to compare measurements obtained in control animals with those obtained in anemic animals.[7] Raw data, as well as data calculated as percent change from baseline data, were analyzed. When significant differences were found, appropriate nonparametric tests were used to identify these differences. A p value ≤ 0.05 was used to indicate significance. In instances of borderline significance we used paired t tests to compare the experimental data with the baseline measurements, both in the control series and in the anemic series. Because several comparisons were being made simultaneously, we used lower p values of ≤ 0.01 to establish statistical significance.

Results

Baseline measurements (shown in Tables I, II, and III) obtained in the animals in the control series did not differ significantly from those obtained in the animals in the anemic series, with two borderline exceptions: Uteroplacental blood flow ($p = 0.05$) and uteroplacental oxygen delivery ($p = 0.07$) were lower in the anemic animals than in the control animals during the control period. Because the 11 animals were distributed randomly into either the control or the anemia series, these differences were most likely a result of sampling (biologic) variability. In the control animals receiving sham transfusions, there were no differences in any of the variables measured when compared with the con-

Table II. Effects of 6 days of anemia on maternal systemic circulation and oxygenation

	Control		*Anemia*	
	Baseline	*After 6-day study period*	*Baseline*	*After 6-day study period*
Cardiac output (ml/min/kg)	137 ± 27	144 ± 20	130 ± 31	184 ± 40*
Maternal heart rate (beats/min)	120 ± 33	106 ± 27	118 ± 12	129 ± 14†
Stroke volume (ml/beat)	71 ± 26	88 ± 30	65 ± 12	85 ± 16*
Mean arterial blood pressure (mm Hg)	108 ± 17	95 ± 16	95 ± 9	94 ± 5
Systemic vascular resistance (mm Hg/ml/min/kg)	0.79 ± 0.26	0.67 ± 0.21	0.74 ± 0.16	0.50 ± 0.08*
Systemic oxygen delivery (ml/min/kg)	16.5 ± 2.5	16.0 ± 3.7	16.3 ± 3.5	10.1 ± 2.7*
Systemic oxygen consumption (ml/min/kg)	7.0 ± 1.3	6.8 ± 1.8	6.8 ± 1.6	5.7 ± 1.4
Systemic oxygen extraction (%)	43 ± 3	45 ± 6	40 ± 5	62 ± 6*
Right ventricular blood P_{O_2} (mm Hg)	45 ± 2	44 ± 3	47 ± 6	36 ± 4*

For control studies $n = 5$; for anemia studies $n = 6$. Values are means ± SD. Stroke volume = cardiac output/heart rate. Note that the baseline data of the control series did not differ significantly from any of the baseline data of the anemia series.

*Significant difference after 6-day study period (anemia) versus baseline measurements.

†Significant difference, anemia versus control.

Table III. Uteroplacental blood flow and oxygenation during chronic maternal anemia

	Control		*Anemia*	
	Baseline	*After 6-day study period*	*Baseline*	*After 6-day study period*
Uteroplacental blood flow (ml/min)	930 ± 114	1034 ± 232	738 ± 196*	1020 ± 177†
Uteroplacental vascular resistance (mm Hg/ml/min)	0.11 ± 0.03	0.09 ± 0.03	0.13 ± 0.02	0.09 ± 0.02†
Uteroplacental oxygen delivery (ml/min)	112 ± 13	118 ± 37	91 ± 29†	58 ± 10†
Uteroplacental oxygen consumption (ml/min)	32.2 ± 1.8	31.1 ± 5.7	27.9 ± 7.3	26.3 ± 4.2
Uteroplacental oxygen extraction (%)	30 ± 4	26 ± 4	33 ± 10	45 ± 4†
Uterine venous blood P_{O_2} (mm Hg)	52 ± 4	54 ± 5	51 ± 6	45 ± 5†
Fetal weight (kg)	—	3.7 ± 0.4	—	3.1 ± 0.3
Uteroplacental weight (kg)	—	1.3 ± 0.9	—	1.3 ± 0.9

For control studies $n = 5$; for anemia studies $n = 6$. Values are means ± SD. Note that all values for blood flow and oxygenation are raw data uncorrected for maternal weight.

*Comparisons of two baseline measurements between control and anemia series were of borderline significance: uteroplacental blood flow, $p = 0.05$; uteroplacental oxygen delivery, $p = 0.07$.

†Significant difference after 6-day study period (anemia) versus baseline measurements.

trol animals that did not receive sham transfusions, and thus those data were pooled.

Table I shows the effects of 6 days of anemia on several hematologic variables. The anemic animals had reductions in their hematocrits and hemoglobin concentrations of about 55%. Because arterial blood oxygen saturations were unaffected by the anemic state, arterial blood oxygen concentration decreased an amount comparable to the hemoglobin reduction (52%). Table II summarizes the maternal systemic response to anemia of 6 days' duration. In the anemic animals cardiac output increased an average of 42%. This increase was the result of increases in heart rate and in stroke volume. Mean arterial blood pressure in the control baseline state was higher than usual at 108 ± 17 mm Hg, because one animal was spontaneously hypertensive. In spite of this degree of hypertension, this animal was included in the data analysis because all of the remaining variables measured were indistinguishable from those of the other control animals. Maternal mean arterial blood pressure was unaffected by anemia, whereas calculated systemic vascular resistance decreased an average of 33%. In spite of the increase in cardiac output, systemic oxygen delivery decreased about 37% during the anemia. Although oxygen delivery decreased, chronic anemia did not affect systemic oxygen consumption, because systemic oxygen extraction increased sufficiently as compensation. The increased oxygen extraction was reflected by a decrease in mixed venous blood (right ventricular blood) P_{O_2}.

Table III summarizes the changes that took place in the uteroplacental circulation in response to anemia of 6 days' duration. In response to anemia, uteroplacental blood flow increased approximately 35%, in association with a 28% decrease in calculated uteroplacental vascular resistance. The increase in uteroplacental blood flow was not sufficient to prevent uteroplacental oxygen delivery from decreasing an average of 35%. In response to this decrease in oxygen delivery, oxygen ex-

traction increased sufficiently for uteroplacental oxygen consumption to be maintained. Associated with the increased uteroplacental oxygen extraction was a reduction in uterine venous blood Po_2.

Comment

The purpose of our experiments was to answer two questions. First, is the uteroplacental adaptation to 6 days of maternal anemia similar to that seen in the systemic circulation? Our data show that maternal anemia produces an adaptation in the uteroplacental circulation that is typical of the systemic circulation as a whole. In both circuits, perfusion increased, although the increase was insufficient to maintain oxygen delivery (Blood flow × Arterial blood oxygen concentration). Without another compensation, both uteroplacental and systemic oxygen consumptions would have decreased. We found that oxygen extraction in both circulations increased sufficiently for oxygen consumption to be maintained. The increases in perfusion of about 35% to 40% were most likely principally a result of reductions in whole blood viscosity induced by the hemodilution. This response is similar to what has been reported for the systemic circulation of the nonpregnant animal[8, 9] where as much as 40% of the increase in systemic blood flow as a result of anemia can be attributed solely to the viscosity-reducing effects of a 50% reduction in hematocrit.[9] Our data clearly show that the increases in uteroplacental perfusion were not the result of increases in perfusion pressure, an important short term regulator of uteroplacental blood flow,[10] because mean arterial blood pressure was unaffected by 6 days of maternal anemia.

Alternatively, it is possible that the increases in systemic and uteroplacental perfusions with anemia were principally the result of active vasoregulation rather than the consequence of reduced viscosity. For example, the drop in mixed venous blood Po_2 that we observed after 6 days of maternal anemia suggests that a potent stimulus for active vasoregulation (decreased tissue Po_2[1, 2] leading to vasodilatation locally or chemoreceptor stimulation peripherally) is present in many vascular beds, in addition to the uterine bed (where uterine mixed venous Po_2 is decreased, Table III). Indeed, the decline in calculated vascular resistance in both the systemic and uteroplacental circulations would seem to support such a view of active vasodilation resulting from 6 days of anemia. Yet this is not necessarily true. The concept of vascular resistance (Mean perfusion pressure/Mean blood flow) as an indicator of vasoconstriction or vasodilation requires, among other things, that hematocrit does not change.[11] When hematocrit varies, resistance to blood flow must be considered by Poiseuille's law to be a function of two components[5]: (1) viscosity, which changes in a predictable manner with anemia, and (2) the cross-sectional area of the various vascular beds, which may or may not change with anemia.[9] Because we did not measure viscosity (the measurement of which is fraught with numerous theoretic and methodologic problems), it is impossible to determine whether any or all vascular beds were involved in active vasoregulation during anemia.

Table IV. Differences in uterine venous and umbilical venous blood Po_2 as function of duration of maternal anemia

	Uterine venous blood Po_2 (mm Hg)	*Umbilical venous blood Po_2 (mm Hg)*
Acute maternal anemia	37 ± 3	18 ± 3
Chronic maternal anemia	45 ± 5*	26 ± 4*

Data on acute anemia are taken from reference 2 ($n = 4$). Data on chronic anemia (6 days' duration) are from unpublished observations made in six near-term pregnant sheep. Values given are means ± SD at maternal hematocrit of 14% ± 1%.

*$p < 0.05$ (unpaired t test), chronic versus acute maternal anemia.

We also wanted to answer a second question: Is the circulatory adaptation to chronic anemia during pregnancy different from that seen with acute anemia? With respect to this question our data indicate that the circulatory response to 6 days of anemia differs both qualitatively and quantitatively from the response to acute anemia in near-term pregnant sheep. With acute anemia we found that cardiac output increased initially as hematocrit was reduced, but at hematocrits <15% cardiac output decreased toward values seen in nonanemic control animals.[3] In the current studies we reduced maternal hematocrit to 14% (or about 50% of control levels) and found that cardiac output was approximately 40% higher than in the control state at the end of the 6-day study period. The difference between the response of cardiac output to acute and chronic anemia suggests that duration of anemia appears to play an important role in the adaptation to the anemic state. It is possible that chronic anemia produces a release of a variety of vasoactive humoral substances, which could lead to long-term increases in perfusion to a variety of vascular beds. It is also possible that the increase in cardiac output is solely the result of reductions in viscosity in the absence of any changes in vascular tone.[5, 9] During acute anemia in nonpregnant animals, for example, reductions in hematocrit of about 50% (similar to those obtained in our studies) lead to reductions in viscosity that are of sufficient magnitude to account for about a 40% increase in perfusion.[9] However, with acute anemia in pregnant sheep, the lack of an increase in cardiac output at hematocrits ≤14% sug-

gests that many vascular beds must have actively vasoconstricted.[3] This predominance of vasoconstriction during acute anemia could have accounted for the lack of increase in blood flow that would have been expected simply as a result of the reduction in apparent viscosity. In contrast, in the chronically anemic state, in which cardiac output increased about 40%, if some regional circulations vasoconstricted during chronic anemia, this response would have to be counterbalanced by vasodilatory responses in other vascular beds to account for the increased cardiac output.

Any difference in the uteroplacental response to acute anemia versus chronic anemia has important implications for the fetus. In response to reductions in hematocrit, tissue perfusion or oxygen extraction or both may increase as a means of adapting to reductions in oxygen availability.[1] When oxygen extraction is used as the principal or sole adaptation to oxygen deficiency in any given vascular bed, the magnitude of the increase in oxygen extraction is reflected by the extent of the reduction in P_{O_2} of the mixed venous blood draining that vascular bed.[2] In the case of the uterine vascular bed, as uteroplacental oxygen extraction increases, uterine venous blood P_{O_2} decreases. Because the placenta of the sheep, as well as that of the human species, functions as a concurrent exchanger,[10] any reductions in uterine venous blood P_{O_2} will be associated with equivalent reductions in umbilical venous blood P_{O_2}.[11-13] Thus any condition that leads to increases in oxygen extraction across the uterine vascular bed will inevitably lead to reductions in umbilical venous blood P_{O_2} (i.e., fetal hypoxemia).[13] During chronic anemia, in contrast to the situation seen with acute anemia, the increase in uteroplacental blood flow that occurs along with the increase in oxygen extraction serves to minimize the reduction in uterine venous blood P_{O_2} that would otherwise result from chronic anemia. Table IV illustrates the implications of this principle. The table provides preliminary data on the uterine venous and umbilical venous blood P_{O_2}s of four animals made anemic for a short time (data from reference 2) compared with six sheep made anemic for a longer time (unpublished data). At a maternal hematocrit of 14% (± 1 SD), both uterine and umbilical venous blood P_{O_2}s were lower with acute anemia than with chronic anemia. Thus, for the same degree of maternal anemia, chronic anemia is associated with less fetal hypoxemia than is acute anemia. This difference in adaptation is due entirely to the augmented uteroplacental perfusion that is associated with chronic anemia but is lacking with acute anemia.

In summary, the current data indicate that the response of the uteroplacental circulation to 6 days of maternal anemia is qualitatively and quantitatively similar to that seen in the systemic circulation. In both circulations, anemia is associated with increases in perfusion and in oxygen extraction to maintain oxygenation. The response to anemia of 6 days' duration differs strikingly from that seen with acute anemia and suggests that duration of anemia plays an important role in the circulatory adaptation to anemia during pregnancy. These results could have important implications for our understanding of the effects of chronic anemia on human pregnancy.

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Analysis of peripheral blood of pregnant women for the presence of fetal Y chromosome–specific ZFY gene deoxyribonucleic acid sequences

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Deoxyribonucleic acid sequences of human ZFY (zinc-finger-Y) gene, a Y-chromosome–specific gene and candidate for the testis-determining factor, has been identified by an in vitro enzymatic deoxyribonucleic acid amplification method in peripheral blood specimens of women pregnant with male fetuses. This technique permits detection of ZFY gene deoxyribonucleic acid sequences in as few as a single male cell among 1,000,000 female cells. Maternal blood results were confirmed by amplification of ZFY gene deoxyribonucleic acid sequences in chorionic villus cells and by karyotyping in 33 of 36 pregnant women. There was no false-positive male result, and two of the three blood specimens with false-negative results were obtained from pregnant women at a very early gestational age. With properly designed guidelines, this deoxyribonucleic acid amplification method may be an alternative to determine the fetal sex for those pregnancies at risk for X-linked genetic disorders. (Am J Obstet Gynecol 1992;166:1013-9.)

Key words: ZFY gene, polymerase chain reaction, peripheral maternal blood, fetal sex determination

Transfer of nucleated fetal cells into maternal circulation in early pregnancy has been a controversial issue in the past.[1, 2] Such a phenomenon is of great interest because fetal cells isolated from maternal blood, obtainable by venipuncture, should be a potentially very valuable source for the prenatal diagnosis of fetal genetic abnormalities. Morphologic observations led the way in early studies on the presence of fetal cells in maternal circulation. Recent reports with monoclonal antibody technologies have suggested that fetal trophoblast cells and nucleated erythrocytes are found in the peripheral blood of pregnant women,[3-5] but the procedures to isolate these cells are laborious and cells recovered are often either too few or not homogeneous enough to be used in conventional biochemical or genetic analysis.

Deoxyribonucleic acid (DNA) amplification catalyzed by thermostable DNA polymerase and directed by flanking oligonucleotide primers is a very powerful method for amplifying extremely small amounts of target DNA.[6, 7] Polymerase chain reaction may provide us with enough sensitivity to identify and analyze fetal genes when the numbers of fetal cells in maternal blood are too few for other types of analyses. There were two major goals in our attempts to develop a DNA amplification protocol to detect fetal DNA sequences from peripheral blood of pregnant women in early pregnancy. Our first goal was to demonstrate unequivocally the presence and abundance of fetal cells in the blood of pregnant women. With a human Y-chromosome–specific gene as a model for our investigation, our second goal was to examine the feasibility of prenatal determination of fetal sex with maternal blood. This objective is important and may become very useful in the management of more than 130 X-linked genetic disorders, such as classic hemophilia and Duchenne and Becker muscular dystrophy. Amplification-based methods have been used in the identification of human Y-chromosome–bearing cells in blood samples from pregnant women bearing male fetuses.[4, 8-12] However, results of these earlier reports were either negative[8] or complicated by a number of things such as a high false-positive rate,[9] autosomal cross-reactivity of the Y-specific primers used,[9, 13] contamination,[9, 11] and previous pregnancies.[14]

The results reported in this study demonstrated that

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cells of fetal origin can be found in the circulating blood of pregnant women in early pregnancy. Our results also suggested that with properly engineered primers and carefully controlled amplification conditions it is feasible to detect the presence of DNA sequences of single-copy genes on fetal Y chromosome and to determine the fetal sex with peripheral blood of pregnant women by our DNA amplification method.

Material and methods

Sample preparations. After informed consent was obtained from subjects, venous blood specimens were collected in Vacutainer (Becton Dickinson, Rutherford, N.J.) blood collecting tubes containing ethylenediaminetetraacetic acid from male and female laboratory staff members and from 36 pregnant women who underwent chorionic villus sampling, because of advanced maternal age, between 8 and 12 weeks of gestation. Blood was always collected before chorionic villus sampling to avoid the possibility of trophoblast cells being released into the circulation as a consequence of the procedure. DNA was purified from chorionic villi and peripheral blood mononuclear cells by phenol and chloroform extraction and recovered by ethanol precipitation before being subjected to DNA amplification.

Amplification of zinc-finger-Y (ZFY) gene DNA sequences. DNA purified from equivalent to approximately 1 ml of whole blood or from two chorionic villi was used for the amplification of ZFY gene DNA sequences with thermostable *Taq* DNA polymerase in a thermal cycler apparatus (Perkin-Elmer Cetus, Norwalk, Conn.). Procedures for the amplification and subsequent detection of amplified DNA were similar to those we have reported earlier,[15-17] with the following modifications to include the nested amplification. Briefly, the 100 μl DNA amplification reaction mixture contains 10 mmol/L Tris hydrochloride, pH 8.3; 50 mmol/L potassium chloride; 1.5 mmol/L magnesium chloride; 0.01% gelatin; 20 pmol each of the two primers; 2.5 nmol each of the four deoxyribonucleotide triphosphates; 1 U of *Taq* DNA polymerase (Perkin-Elmer Cetus), and specimen (positive) or control (negative) DNA. The temperature of the reaction mixture was first raised to 94° C for 1 minute to denature the DNA and then cooled down to 55° C for another minute to allow annealing of primers to putative target DNA sequences. This procedure was followed by raising the temperature to 72° C for 1 minute to extend the DNA chain. The whole process was repeated 32 times with a 10-minute incubation at 72° C at the end. The nested polymerase chain reaction uses a second set of primers located inside the DNA, flanked by the initial or first set of primers, and uses the amplified products resulting from the first amplification as a template. The nested amplification both increases the overall sensitivity and enhances the specificity of polymerase chain reaction by minimizing false-positive results. For the nested amplification of ZFY gene DNA sequences, 2 μl of the first amplified mixture was subjected to the same amplification procedures, except for 40 cycles in a freshly prepared 100 μl reaction mixture containing the same components as the first polymerase chain reaction and the new set of nested primers. The sequences of primers were derived from DNA sequences of the human ZFY gene which is located on the Y chromosome and which is a candidate for the testis-determining factor and has been suggested to be associated with the initiation of testis differentiation during embryogenesis.[18] The selection of oligonucleotide primers was performed with a computer program for efficient amplification by polymerase chain reaction.[19] Sequences of the first set of primers (from 5′-end to 3′-end), which flank a 354-base-pair region of the human ZFY gene on the Y chromosome, are CCATTTGTTCTAAGTCGCCA (2125 nucleotide) and CTTCAAGGCCAACATCAGCTG (2478 nucleotide). The sequences of the nested primers are CTCTCAGTTCACACAAAGG (2149 nucleotide) and GCTTGTAGACACACTGTTAGG (2455 nucleotide), which flank a 307-base-pair region located inside the primers used in the initial amplification.

Establishment of human ZFY gene DNA sequence positivity. After the nested amplification, one tenth of the amplified reaction mixture was separated by electrophoresis in 2% agarose gel containing 0.5 μg/ml ethidium bromide and visually inspected under ultraviolet light for a DNA band of 307 base pairs to determine the human ZFY gene DNA sequence positivity. The amplified ZFY gene DNA was further analyzed by restriction endonuclease digestion and by Southern blot DNA hybridization with an internal oligonucleotide probe. The internal oligonucleotide (TTCATACAAAAGACTATCCTCATCGGTG) was labeled at its 5′-end with γ-phosphorus 32-adenosine triphosphate by polynucleotide kinase to approximately 5×10^6 counts/min/pmol of oligonucleotide and used for Southern blot analysis. Procedures for Southern blot hybridization have been described previously by others[20] and were modified for oligonucleotide probes as follows: After hybridization, the filters were washed sequentially in 2× SSC (1× SSC is 0.15 mol/L sodium chloride and 0.015 mol/L sodium citrate) and 0.5% sodium dodecyl sulfate twice for 10 minutes each at room temperature and then in 0.1× SSC and 0.5% sodium dodecyl sulfate three times for 20 minutes each at 55° C. Restriction endonucleases were purchased

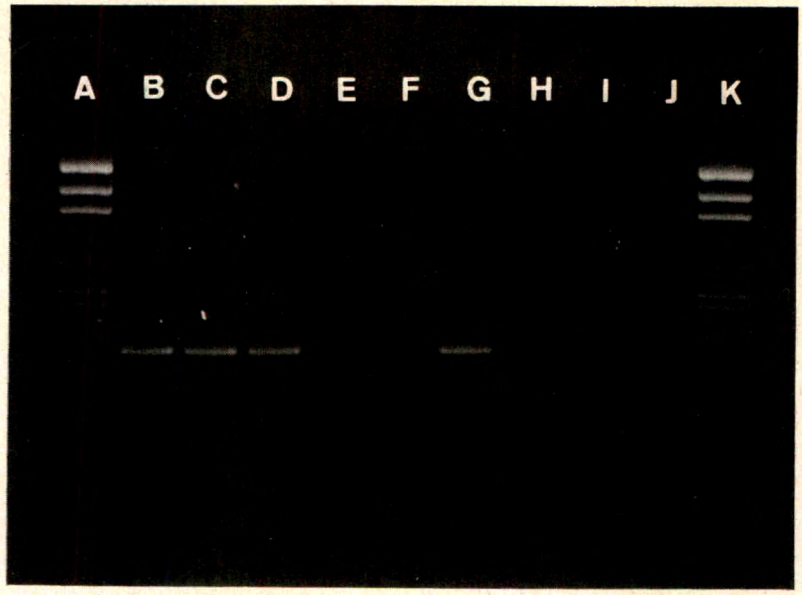


Fig. 1. Detection of ZFY gene DNA sequences by DNA amplification. Sequences of primers and procedures for sample preparation and amplification are described in Material and methods. pGEM-3 DNA digested with a mixture of restriction endonucleases *Hin*fI, *Rsa* I, and *Sin* I was used in *lanes A* and *K* as size standards, and sizes are (from top to bottom) 2645, 1605, 1198, 676, 517, 460, 396, 350, 222, 179, 126, 75, 65, 51, and 36 base pairs. *Lanes B, C,* and *D* are amplifications of 1 ng and 100 and 10 pg of human genomic DNA prepared from peripheral blood mononuclear cells of a male volunteer, equivalent to approximately 200, 20, and 2 cells, respectively. *Lanes E* and *F* are amplifications of 10 and 1 μg of genomic DNA prepared from cells of a female volunteer, equivalent to approximately 2×10^6 and 2×10^5 cells, respectively. *Lanes G* and *H* are amplifications of DNA prepared from chorionic villus cells obtained from women who bore male *(lane G)* or female *(lane H)* fetuses. *Lanes I* and *J* are amplifications of 1 ng bacterial cell *E. coli* and 50 ng mouse DNA, respectively.

from either Boehringer Mannheim (Mannheim, Germany) or BRL (Gaithersburg, Md.) and used according to the manufacturers' recommendations.

Precautions against contamination and false-positive results in polymerase chain reaction. Because of the exquisite amplification power of the polymerase chain reaction procedures, precautionary measures were necessary to minimize any possibility of contaminations including sample-to-sample contaminations and polymerase chain reaction product carryovers. These measures include wearing and frequent changing of disposable gloves during all procedures that involve specimen handling and processing, aliquoting of all reagents, physical separation of locations for pre– and post–polymerase chain reactions, use of closed-end blood collection tubes (Vacutainer), and meticulous laboratory techniques. Because of the nature of the target DNA, all experimental procedures in this study were performed by female staff members of the laboratory. Furthermore, all reagents were irradiated with ultraviolet light to inactivate any contaminating double-stranded DNA that may have been present before sample DNA was added and the polymerase chain reaction started.[21] Fifty nanograms of human female DNA (from equivalent to approximately 7500 cells) or 1 ng of *Escherichia coli* DNA (equivalent to approximately 200,000 bacterial cells) was used as a negative control that always yielded negative results. Multiple reagent controls were also included in each polymerase chain reaction assay, giving negative results. Repeated DNA amplification assays of the same specimens at different times or performed by different laboratory members produced the same results.

Results

The amplification of ZFY gene DNA sequences flanked by the primers used in this study is indicated by the presence of the 307-base-pair DNA after nested amplification (Fig. 1). The sensitivity and specificity of this amplification procedure were determined by the amplification of serial dilutions of human genomic DNA of known concentrations prepared from peripheral blood mononuclear cells of male and female individuals. As few as 10 pg of genomic DNA from equiv-

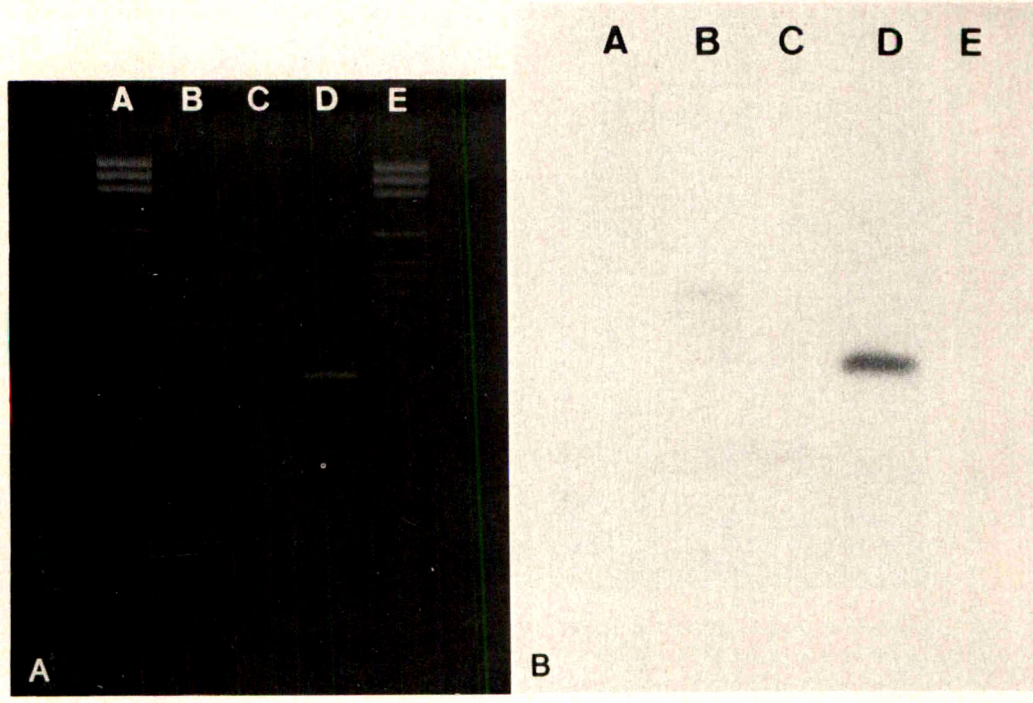


Fig. 2. A, Restriction endonuclease digestion of amplified ZFY gene DNA. *Lanes A* and *E* are same DNA size standards used in Fig. 1. *Lane B* is 307-base-pair ZFY gene DNA resulting from nested amplification. *Lanes C* and *D* are amplified ZFY gene DNA after restriction endonuclease *Mae* II and *Nde* I treatment, respectively. **B,** Autoradiograph of Southern blot hybridization of amplified ZFY gene DNA with internal oligonucleotide probe. DNA shown in *panel A* was transferred onto nitrocellulose membrane filter and hybridized to ^{32}P-labeled oligonucleotide probe as described in Material and methods. Exposure time was 25 minutes at room temperature without intensifying screen.

alent to approximately fewer than two peripheral blood mononuclear cells of a male subject consistently produced a clear positive signal (Fig. 1, lane D). Negative results with practically no visible background smears were obtained with the amplification of ≥10 μg DNA obtained from equivalent to approximately 2 million peripheral blood mononuclear cells of a female subject (Fig. 1, lane E). Chorionic villus cells from one woman who bore a male fetus and one who bore a female fetus produced positive and negative results, respectively (Fig. 1, lanes G and H). The primers used in this study do not cross amplify DNA of bacterial *E. coli* or mouse cells (Fig. 1, lanes I and J).

The specificity of amplification and the authenticity of the amplified DNA were further evaluated by two additional and independent methods. First, the putative amplified ZFY gene DNA was subjected to digestion by restriction endonucleases. When the 307-base-pair DNA generated by the nested amplification (Fig. 2, *A*, lane B) was treated with restriction endonuclease *Mae* II or *Nde* I, the original DNA disappeared and was replaced with DNA fragments of 168 and 139 base pairs (Fig. 2, *A*, lane C) or 223 and 84 base pairs (Fig. 2, *A*, lane D), respectively. The sizes of these DNA fragments match precisely with the sizes deduced from known DNA sequences and sites of the restriction endonuclease. The specificity of the amplification was further confirmed by Southern blot hybridization with an internal oligonucleotide probe. The 307-base-pair putative human Y-chromosome DNA and the 139- and 223-base-pair DNA fragments generated by *Mae* II and *Nde* I restriction endonuclease digestion, respectively, hybridized with the internal oligonucleotide probe (Fig. 2, *B*). These results are also in complete agreement with the locations of the *Mae* II and *Nde* I restriction sites with respect to that of the probe sequence.

The ability of the primers and amplification protocols used in this study to identify extremely small numbers of male cells in the presence of large numbers of female cells was demonstrated by mixing female and male cells at different ratios. Amplified ZFY gene DNA could be visualized directly in agarose gel stained with ethidium bromide when DNA was prepared from a mixture containing a few male cells and 1×10^6 female cells (Fig. 3, lanes B to E). The actual amplification of ZFY gene DNA sequences from peripheral blood of pregnant women was illustrated with the blood of two women who bore male fetuses (Fig. 3, lanes F and G) and one woman with a female fetus (Fig. 3, lane H).

Finally, the results of the amplification of ZFY gene

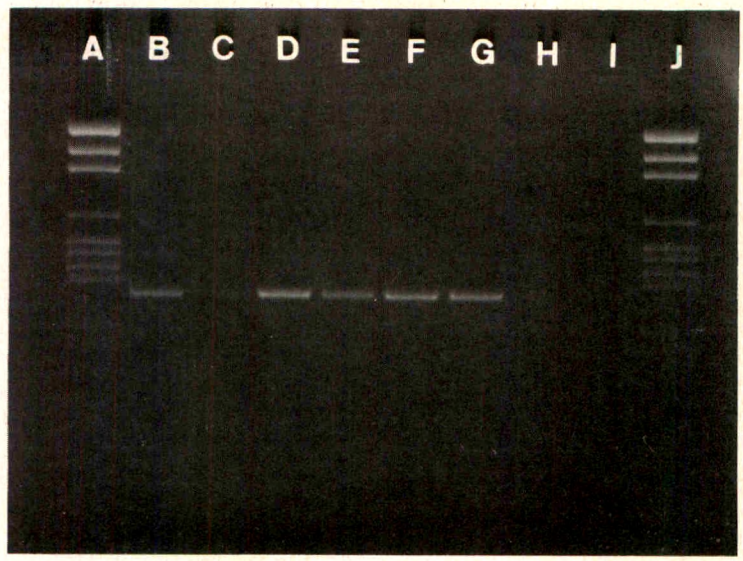


Fig. 3. Amplification of ZFY gene DNA sequences with DNA prepared from mixture of peripheral blood mononuclear cells of male and female fetus at different ratios. DNA size standards in *lanes A* and *J* are same as described in legends to Figs. 1 and 2. *Lanes B* and *C*, 10^6 Female cells mixed with 10 and 1 male cells, respectively. *Lanes D* and *E*, 10^5 Female cells mixed with 10 and 1 male cells, respectively. *Lanes F* and *G* are amplifications of ZFY gene DNA sequences of DNA prepared from peripheral maternal blood specimens of two pregnant women carrying male fetuses. *Lane H* is from woman carrying female fetus. *Lane I* is DNA-negative control.

DNA sequences from peripheral blood of 36 pregnant women were compared with those of the amplification of ZFY gene DNA sequences of chorionic villus cells and with karyotyping (Table I). Twenty and 13 pregnant women were found to be positive and negative, respectively, for the presence of ZFY gene DNA sequences in both peripheral blood and chorionic villus cells. Amplification of ZFY gene DNA sequences in chorionic villus cells agreed with the results of karyotyping in all 36 cases. Three women showed Y-chromosome DNA sequences in chorionic villus cell amplification but not in blood specimens, even with up to five times the starting amount of DNA. Two of these three women were at <8 weeks of gestation at the time the chorionic villus sampling and blood specimens were taken. All 20 blood specimens in Y-chromosome DNA–positive patients were confirmed by the presence of Y-chromosome DNA sequences in their chorionic villus cells and also by karyotyping. Four babies have been born so far, all male, and all four have been identified earlier by the amplification of Y-chromosome DNA sequences with the blood of the mothers during pregnancy.

Table I. Amplification of human ZFY gene DNA sequences in chorionic villus cells and maternal blood of pregnant women

Results of chorionic villus sampling	*Results in maternal blood*	
	Positive	*Negative*
Positive	20	3
Negative	0	13

Comment

Identification of fetal cells and determination of the presence of Y chromosome–specific DNA sequences in the peripheral blood of pregnant women in early pregnancy can be very valuable for individuals at risk for recessive X-linked disorders that typically affect only hemizygous male subjects. In some of these disorders prenatal diagnosis is possible by chorionic villus sampling or amniocentesis followed by cytogenetic, biochemical, or molecular analysis of the recovered fetal cells. Because the gene or genes responsible for most of the recessive X-linked disorders have not yet been identified, the only other alternative to prenatal diagnosis in these cases is to determine the gender of the fetus. If the fetus is either affected by the genetic defect or of male gender, termination of pregnancy can be an option. Standard karyotyping and cytogenetic analysis of chorionic villus cells or amniocytes to detect the Y chromosome depend on obtaining multiple high-quality metaphases from cells cultured for days to weeks before analysis. Maternal blood combined with DNA amplification may be an alternative that is much faster, more accessible, and even safer than the current invasive chorionic villus sampling and amniocentesis.

Polymerase chain reaction DNA amplification has correctly determined the presence of the Y-chromosome–specific DNA sequence in single cells obtained from in vitro fetilized, preimplantation human embryos,[22] which has led to the successful transfer of female embryos to mothers who were carriers of serious X-linked disorders.[23] Various reports have put the numbers of fetal cells in maternal blood at one fetal cell in every 1000 to 40,000 maternal cells.[4, 5, 10] On the basis of these estimations and judging from the capability of our method to detect one male cell in 1,000,000 female cells (Fig. 3), the primers and protocols used in our study should be able to pick up Y-chromosome DNA sequences in circulating maternal blood of women pregnant with male fetuses. Enrichment of fetal cells in maternal blood by monoclonal antibodies[9] or other means does not seem necessary for the detection of Y-chromosome DNA sequences.

Blood specimens of two of the three women with positive results for ZFY gene DNA sequences in chorionic villus cells but negative results in blood were obtained at a very early gestational age (<8 weeks). Blood specimens from the same two women were positive when tested again at 10 and 11 weeks' gestation, respectively. This observation seems to suggest that the gestational age may be an important factor in determining the availability of fetal cells after transfer to the maternal circulation. If only blood specimens from pregnant women at ≥8 weeks' gestational age were tested, the current method was correct in determining the fetal sex in 33 of 34 cases. The absence of any false-positive result, however, is a great asset because this test will probably be used mainly to identify male instead of female fetuses in pregnancies at risk for X-linked recessive genetic disorders. An excellent positive predictive value of the test will assure identification of all that may be affected by the genetic marker. Other advantages of this test over karyotyping or DNA probe hybridization of chorionic villus cells or amniocytes are that this test can be performed much earlier than amniocentesis and that the tests can be completed in less than a day because the automated DNA thermal cycler instrument does most of the work and does not require isotopes.

There are a number of restrictions before the current method can be used for routine screening purposes. Strict but workable guidelines to control the problem of contamination need to be explored and investigated, including precautions generally applicable to all polymerase chain reactions and specific ones to guard against false-positive results with regard to Y-chromosome–specific sequences.[24] Other issues, such as the effect of previous pregnancies and the occasional presence of Y-chromosome–specific sequences in XY female subjects,[12] also need to be examined. For this and other similar amplification-based methods used for the prenatal diagnosis of genetic disorders, separation of fetal from maternal cells is probably needed[3, 4] because only then could fetal or maternal origin of a given genetic disease marker be distinguished. As the method exists now, only sex determination and paternally derived disease marker analysis are feasible. In any event prenatal diagnosis of increasing numbers of genetic disorders in early pregnancy with maternal blood and preimplantation embryos is becoming available.

In summary, we have shown that with carefully engineered primers, well-designed protocols, and strictly controlled anticontamination measures the detection of ZFY gene DNA sequences and determination of fetal sex with maternal blood obtained in early pregnancy is possible.

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First report: Telemetry-assisted ambulatory nonstress test to evaluate umbilical cord compression

To the Editors: The resting nonstress test is performed with the pregnant patient supine and inactive. Fetal heart rate accelerations in association with fetal movement suggest fetal well-being. In addition to accelerations the baseline fetal heart rate should decline toward term from 150 to 120 beats/min.[1] But what about the patient outside the office who is active, ambulatory, and erect? To evaluate this influence, two patients with suspected umbilical cord compression (based on 36 weeks' fetal heart rates of 140 to 150 beats/min) were assessed in both the supine and erect positions. The classic nonstress test differed from the ambulatory nonstress test. A telemetry unit (model no. 80240A, Hewlett Packard, Palo Alto, Calif.) recorded active and resting fetal heart rates. Obvious differences were observed between the two tests (Fig. 1). Continued observation with telemetry and hospitalization of these patients between 36 and 38 weeks' gestation verified the heart rate changes. Both patients were delivered of infants with umbilical cord entanglements and related cord compression. One patient also had a vas insertion of the cord.

The supine-inactive nonstress test may give mislead-

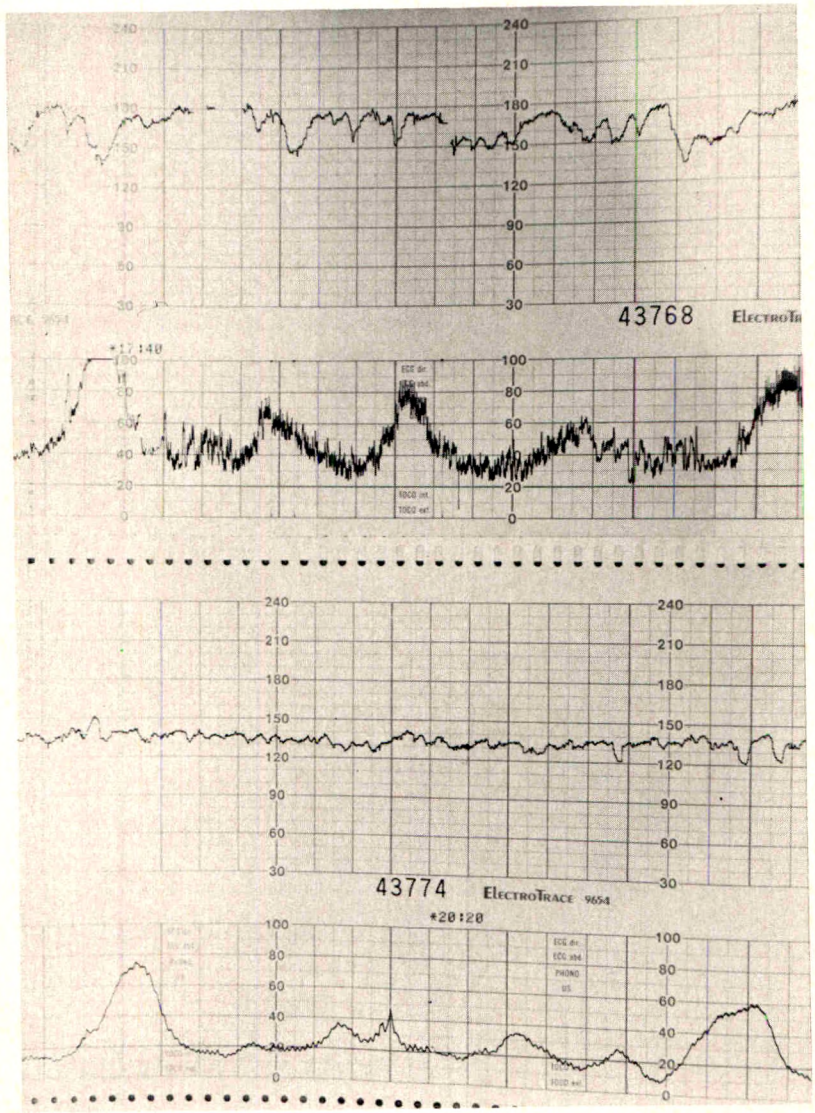


Fig. 1. Nonstress tests of patient A at 37 weeks' gestation recorded with telemetry during ambulation (*top*, 43768) and supine rest (*bottom*, 43774).

ing results in borderline cases of fetal compromise. Clinically suspected compromised fetuses can go undetected, with supine nonstress tests showing fetal acceleration with movement. A compromised fetus may be detected with a telemetry-assisted ambulatory nonstress test.[2]

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Frequency of positive antinuclear antibody test results in women with recurrent spontaneous abortions

To the Editors: The recent article by Xu et al. (Xu L, Chang V, Murphy A, et al. Antinuclear antibodies in sera of patients with recurrent pregnancy wastage. AM J OBSTET GYNECOL 1990;163:1493-7) from The Johns Hopkins Hospital suggests that women with "unexplained" consecutive pregnancy losses are much more likely (40%) than controls (pregnant 8.2%, nonpregnant 5.6%) to have a positive antinuclear antibody test result. This proportion of positive antinuclear antibody test results is markedly higher than that found by most others in the literature.[1,2] We recently published a prospective, controlled study of antinuclear antibody tests in 277 women with recurrent pregnancy losses and found that 16.3% of cases had a positive titer at ≥1:40.[2] In 299 pregnant controls, however, 16.6% had a positive antinuclear antibody test result, and 16.8% of 119 nonpregnant control patients had a positive antinuclear antibody test result. Our results agree with those of Petri et al.,[3] who found positive antinuclear antibody test results (using HEp-2 cells) in 16% of cases compared with 20% of controls. These results are quite different from those of Xu et al. Such differences in clinical studies can often be explained by differences in patient selection. Xu et al. reported on results from 30 women with "explained" pregnancy losses and 30 with "unexplained" pregnancy losses over a 39-month period. The neat matching of these two numbers suggest that some patient selection was involved, although the authors did not address that point in their Material and Methods section. Did the 30 patients in their group A constitute the entire population of patients with unexplained recurrent pregnancy losses seen from August 1984 to November 1987? Did the 30 patients in their "explained pregnancy loss" group constitute a remarkably coincidental number of explained recurrent pregnancy losses? If not, how were the patients in these groups chosen?

Since they apparently used a technique and target cells (HEp-2) similar to ours, can they explain this striking difference in which nearly half of the 60 women had a positive antinuclear antibody test result? If the proportions are correct, this difference suggests that a positive antinuclear antibody test result does not discriminate between patients with recurrent pregnancy losses, whether their pregnancy losses are explained (53.3% positive test result) or unexplained (40% positive test result). Did the authors conclude therefore that the "explanation" for the pregnancy losses is really not entirely correct?

Finally, the authors suggested that "the screening test for antinuclear antibodies in patients with recurrent fetal wastage should be considered." While that may be true, a single antinuclear antibody test is not a satisfactory screening procedure for autoimmune disease. Rather one must ask questions about a history of signs and symptoms to ascertain the presence of autoimmune disease.

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Reply

To the Editors: We thank Dr. Harger for his comments and careful review of our recent article, but we also wish to respond to the issues that he raises in his letter. Our study began with a review of 60 patients with repeated pregnancy losses who had completed an evaluation while under our care between August 1984 and November 1987. The frequency of unexplained pregnancy losses is quite high, having been reported to be 37% to 44%[1,2]; therefore we were not surprised at finding a 50% rate of unexpected losses in our patient population, especially when this was what we had already observed from clinical experience before 1983.[3] No attempt was made to preselect or match cases.

We, too, were surprised at the high frequency of low-positive antinuclear antibody titers in our pregnancy loss groups, but we were most reassured after comparable results for antinuclear antibody titers were obtained from two different laboratories on the same blood samples. Furthermore, the frequency of low-positive antinuclear antibody titers noted in our nonpregnant group (5.6%) agreed with data from Fritzler et

al.,[4] who assayed sera taken from 2500 female blood donors.

One conclusion that can be drawn from our study is that low-titer antinuclear antibody sera might reflect repeated losses rather than serve as a *cause.* Alternatively, low-positive-titer antinuclear antibody sera may represent a "cross-reaction" for an as yet unidentified immunologic cause of pregnancy losses. For these reasons we believe that antinuclear antibody screening *should* be considered for patients with repeated pregnancy losses.

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Prophylactic sacrospinous fixation discouraged

To the Editors: I strongly object to your publication in the Clinical Section of Cruikshank's article (Cruikshank SH. Sacrospinous fixation—Should this be performed at the time of vaginal hysterectomy? AM J OBSTET GYNECOL 1991;164:1072-6).

This article appeared in a previous issue of your JOURNAL.[1] It was presented to the Central Association of Obstetricians and Gynecologists and it received a full discussion (which was also published) by Dr. George Morley. Dr. Morley pointed out that the prophylactic performance of sacrospinous ligament fixation of the vaginal vault during hysterectomy in women with the potential for pelvic relaxation is unwarranted. The authors report that the incidence of vaginal vault prolapse after hysterectomy ranges from "2 to 45%." Certainly most authorities, including these authors in their previous publication, would agree that this incidence is highly overstated.[1-4] Moreover, in most reported series the incidence is certainly below 5%.[1-4]

Other complications have been reported variously, including two deaths from this procedure.[1, 5] Sacrospinous ligament fixation is not an innocuous procedure and should not be advocated for prophylaxis. Moreover, this procedure should be performed only by those who have had specialized training in its performance, as it poses several other risks, including but not limited to sciatic nerve injury, segmental nerve loss, and profuse bleeding.

I therefore object to the publication of this Clinical Opinion based on the experience of one author. Perhaps in Dr. Cruikshank's hands this operation goes smoothly, as he states in his previous article with an average operating time of 18 minutes.[1] To translate his facility in this procedure to advocacy of its usage by other practitioners with varying surgical skills may be dangerous.

In conclusion, I do not agree with the use of vaginal vault sacrospinous ligament fixation in patients with potential vault prolapse or potential pelvic weakness and would agree with Dr. Morley in his previous criticism that Dr. Cruikshank's position is certainly not warranted and may be downright dangerous.

I appreciate the opportunity to express this opinion and welcome the author's comments.

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Reply

To the Editors: I appreciate the interest Dr. Scotti has shown in my article. I will answer his questions individually.

Prophylaxis means to prevent, to ward off. It *does not* mean routine. This has been misinterpreted. After several letters and a formal request to possibly publish a Clinical Opinion, I felt that I should do so, to clear up this misunderstanding. I want to reiterate, as I published in *Surgical Profiles,* January 1991 (Merck Sharp and Dohme), that only 8% of the patients in my own population met the criteria for this adjunct fixation. The number increased to 35% when outside surgical referrals were added. The 43% incidence of vault prolapse noted in the original article on sacrospinous fixation stems from the 1960s literature.

I am fully aware that sacrospinous fixation is not an innocuous procedure and should not be a *routine* procedure. Prophylaxis is not routine. All defects of the vagina should be corrected at the initial operation or failure and complications can occur. Bleeding, nerve injury, etc., are serious complications, and some patients die. Before acceptance of Dr. Scotti's article on massive small bowel evisceration at the time of sacrospinous fixation, as a reviewer I asked these questions: Were the patients evaluated for multiple defects and were these corrected at the initial surgery? Was

there a missed enterocele? Had the vaginal vault been closed? Prevention is the key that we strive to achieve.

This Clinical Opinion was not based solely on my experience. After supportive letters and an invitation to write this Clinical Opinion, I saw the need. Those wishing to learn this procedure should do so from experienced teachers; they should learn the anatomy and the pelvic floor defects in patients with these kinds of problems. I believe that every patient should be *thoroughly* evaluated for all defects. These should be corrected at the time of hysterectomy, not as a piecemeal procedure whereby the patient will have to undergo several operations.

In conclusion, I say that the sacrospinous ligament fixation is a good adjunct procedure in patients who have multiple defects of the vaginal wall. I have described criteria for patients at risk for vaginal vault prolapse as a result of these defects. When learned appropriately, under the right supervision, sacrospinous fixation at the time of hysterectomy can be a perfect adjunct to the surgical armamentarium.

Again, I thank Dr. Scotti for his comments.

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Polyunsaturated fatty acids in preeclampsia

To the Editors: The interesting article of Wang et al. (Wang Y, Kay HH, Killam AP. Decreased levels of polyunsaturated fatty acids in preeclampsia. AM J OBSTET GYNECOL 1991;164:812-8) deals with the present problem of polyunsaturated fatty acids and hypertension, in relation to preeclampsia. This is an aspect that has hitherto received little attention. The authors found significantly lower levels of polyunsaturated acids and of linoleic and eicosapentaenoic acids and suggest that the reduced levels of the latter may play a significant pathophysiologic role in preeclampsia.

In a study performed in Spain as early as 1979,[1] in 14 women with preeclampsia and their newborn infants and 14 control women and their newborn infants, we found significantly lower levels of linoleic acid among women with preeclampsia (29.4% vs 23.3%), as Wang et al. report. Unfortunately the laboratory techniques at that time did not permit determination of *n*-3 eicosapentaenoic and docosahexaenoic fatty acids. However their precursor, linolenic acid (reduced in the work of Wang et al.), showed no differences.[1]

However, our work did present some facts about polyunsaturated fatty acids in newborn infants of preeclamptic mothers that may be worth reporting now. The aforementioned differences in linoleic acid among the mothers were found, but there were no differences in linoleic acid or in the other fatty acids analyzed among newborns from preeclamptic mothers and those from controls. There was only one newborn infant whose mother had a very low level of linoleic acid and the umbilical cord level of linoleic acid was at the lower limit of normality.

Nevertheless, bearing in mind the significance of linoleic acid as a precursor of more elongated *n*-6 fatty acids, which are necessary for brain development and prostanoid synthesis,[2] we think linoleic acid should receive more attention in the study of the pathophysiologic features of preeclampsia. In accordance with this, the remarkable difference in total polyunsaturated fatty acids reported by Wang et al. depends fundamentally on the difference on linoleic acid.

In summary, we think that, without underestimating *n*-3 fatty acids, the *n*-6 class should receive more attention. Finally, we would like to put a question to the authors, in the light of their relevant findings and ours: Do they think that reduced fatty acid levels are primarily of maternal origin, with some kind of active transfer to the fetus, or could it be that there is an increased fetal consumption in preeclamptic pregnancies?

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Reply

To the Editors: Sanjurjo et al. pose an interesting question. It is not known whether the decreased levels of fatty acids we found in the preeclamptic patients in our study are due to maternal factors (i.e., decreased intake, decreased mobilization from body stores, or increased metabolism) or to increased fetal consumption. Since preeclampsia is a pregnancy-related disease, a fetal factor certainly may be important. Essential fatty acids, which include linoleic and linolenic acids, are important for fetal growth and development because they are incorporated into phospholipids that are necessary for cell membrane synthesis. They are known to be transported across the placenta in increasing amounts with advancing gestation. Cord blood levels of linoleic acid are reported to be lower than maternal levels. The relatively lower level of linoleic acid in fetal blood facilitates maternal-fetal transport across the placenta.[1] The reason for a possible increase in the transport across the placenta or for an increase in fetal requirements for these polyunsaturated fatty acids in preeclampsia is not known and would require further investigation.

There is, in fact, another possible drain on these maternal fatty acids and that would be the placenta, which is known to synthesize and metabolize large amounts of eicosanoids. It has been shown that placental production of thromboxane is actually increased in preeclampsia.[2] Since linoleic acid is a precursor of thromboxane, it would be possible to explain the decreased maternal plasma levels of linoleic acid by the enhanced synthesis of this vasoconstrictor in the pla-

centa. We agree with the need for further studies on these important fatty acids.

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Sex selection and early pregnancy fetal sex determination: Ethics and personal freedom of choice

To the Editors: This letter pertains to an article in the April 1991 issue of the JOURNAL (Evans MI, Drugan A, Bottoms SF, et al. Attitudes of the ethics of abortion, sex selection, and selective pregnancy termination among health care professionals, ethicists, and clergy likely to encounter such situations. AM J OBSTET GYNECOL 1991;164:1092-9).

I would like to point out an "apparent change of heart" in the ethical perspective of John C. Fletcher, PhD, formerly of the National Institutes of Health, who wrote in the Sounding Board section of *The New England Journal of Medicine*, Sept. 6, 1979, page 550, in the article "Ethics in amniocentesis for fetal sex determination, and whom I quoted previously[1]:

> I have reevaluated my position on this issue as a result of participation in the Hastings Center study group in consultation with the staff from the prenatal diagnostic centers of The Johns Hopkins Hospital and their policy on amniocentesis for sex choice. In my revised view, it is not ethically required that physicians withhold amniocentesis (*or for that matter, new reproductive technologies such as Fetal Sex Determination in Early Pregnancy by FASA,** italics added) from fully informed parents who may use the results in deciding to abort for sex choice. Even though the physician may personally disapprove of the request (*I have personally and publicly stated that my personal private preference is not in favor of abortion using Fetal Sex Determination in Early Pregnancy by FASA for female fetal sex "feticide,"* italics added), it is fairer to the parents to grant it, within the limits of availability of amniocentesis (*or other new reproductive technologies such as FASA*, italics added). Physicians who agree with the social, ethical perspective that informs the legal rules on abortion will finally want to keep faith with the moral intent of the law. Policy makers in this field should now reconsider their obligation to be responsive and consistent in their beliefs. I include myself in the company of those who need to be changed.

The implications of the change of ethical principles in the 1991 article are directly opposite the 1979 writings of John C. Fletcher, PhD, and I wonder how it is possible to explain such a complete reversal in published ethical principles.

*FASA (Fetal Anatomic Sex Assignment) is a service mark of John D. Stephens, MD.

It is my opinion that the only issue that is the sole responsibility of physicians really is support of the patient's (and it is usually women who are burdened with this responsibility) right to exercise her (or, in the instance of a couple in a counseling situation, their) reproductive options, regardless of the indication, regardless of the personal moral or ethical standpoint held by the practicing physician.[2-6] I have previously stated my own personal perspectives on all aspects of this subject.[1,5-8] Quite clearly, genetic counseling to people of a "traditional society" requires sensitivity. Borgaonkar[9] stated: "I strongly support the suggestion that genetic counselors take into consideration societal factors in conducting premarital or preconceptual counseling, not only in a traditional society but also in any society."

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Reply

To the Editors: Dr. Stephens presented a long quotation from one of us (J.C.F.), dated Sept. 6, 1979. In 1979, Dr. Fletcher said that whereas he had previously been opposed to prenatal diagnosis specifically for sex selection, he thought at the time that physician preferences should take a back seat to patient desires. Over the course of the past decade, however, there has been a restabilization, with the pendulum swinging from complete physician authority, through to patient autonomy, and now settling back in again somewhere in the middle. The results of our survey of more than 700 professionals suggest that whereas the majority of this group is ethically accepting of the notion of termination of pregnancy, there is, in general, significant resistance to the use of such technology for sex selection as the only issue. Note that these are *ethical* questions, not legal ones. It is perfectly consistent to be of the opinion that something is unethical but yet should still be legal. Such

is the general interpretation of our own data. Speaking as authors rather than as summarizers of the data, we believe that sex selection crosses the line between the use of technology for genetic reasons and its use for eugenic reasons. We believe that no physician need participate in any action for which he or she feels uncomfortable. Such is different from saying that a patient cannot have such a service or supporting refusal to provide a referral. We find it difficult to understand how Dr. Stephens can accept Dr. Fletcher's change of position from that in the 1979 reference but now doesn't understand how that position could again be changed. Ethics evolve with technology and experience. Our writings speak for themselves.

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Books received

A Challenge to Care: Strategies to Help Chemically Dependent Women and Infants. Edited by The National Organization for Obstetric, Gynecologic and Neonatal Nurses. Running time 38 minutes. Cambridge, Massachusetts, 1990, Vida Health Communications. $275 (videotape).

A Practical Guide to Chorion Villus Sampling. Edited by David T.Y. Liu. 158 pages, illustrated. New York, 1991, Oxford University Press. $62 (hard cover). $32 (soft cover).

Achievements in Gynecology 1989-90. Volume 18. Edited by S. Mancuso and P.J. Keller. 185 pages. Basel, 1991, S. Karger AG. $144 (hard cover).

Actualites gynecologiques. Edited by Albert Netter. 286 pages. Paris, 1991, Masson Editeur. No price listed.

The Baby Doctors. Probing the Limits of Fetal Medicine. Edited by Gina Kolata. 213 pages. New York, 1990, Dell Publishing. $4.99.

Basic and Clinical Biostatistics. Edited by Beth Dawson-Saunders and Robert G. Trapp. 329 pages. East Norwalk, Connecticut, 1990, Appleton & Lange. $29.95.

Echographie en Pratique Obstetricale: Collection D'Imagerie Radiologique. Edited by B. Guerin, Y. Ardaens, B. Bailleux, and D. Houze. 196 pages, illustrated. Paris, 1991, Masson Editeur. No price listed (hard cover).

The Family Guide to Mental Health. Edited by Benjamin B. Wolman. 301 pages. New York, 1991, Prentice Hall General Reference. $35.

Genetics in Medicine. Fifth edition. Edited by Margaret Thompson, Roderick R. McInnes, and Huntington Willard. 512 pages, illustrated. Philadelphia, 1991, W.B. Saunders Company. $37.

Growth Factors in Fertility Regulation. Edited by Florence P. Haseltine and Jock K. Findlay. 380 pages. New York, 1991, Cambridge University Press. $120 (cloth).

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Infertility. Perspectives From Stress and Coping Research. Edited by Annette L. Stanton and Christine Dunkel-Schetter. 240 pages. New York, 1991, Plenum Press. $35.

Labour Ward Manual. Second edition. Edited by D.T.Y. Liu and D.V.I. Fairweather. 131 pages, illustrated. Oxford, 1991, Butterworth Heinemann. $45.

Malignancies of the Vulva. Edited by P.G. Knapstein, F. di Re, P. DiSaia, U. Haller, and B.-U. Sevin. 175 pages, illustrated. New York, 1991, Georg Thieme Verlag. No price listed (hard cover).

Managing the Side Effects of Chemotherapy and Radiation. Edited by Marylin J. Dodd. 209 pages. New York, 1991, Prentice Hall Press. $12.95.

The Merck Manual of Diagnosis and Therapy. Fifteenth edition. Robert Berkow, Editor-in-Chief. 2696 pages. Rahway, New Jersey, 1987, Merck Sharp & Dohme Research Laboratories. No price listed.

Nursing Your Baby: Fully Updated for the 90's. Edited by Karen Pryor and Gale Pryor. 416 pages. New York, 1991, Pocket Books. $5.95.

Obstetrics & Gynecology Review, 1991: Ideal for Board Review. Edited by Harrison H. Sheld. 435 pages. New York, 1991, Pergamon Press, Inc. No price listed.

Pathways to Parenthood. Improving Your Chances of Pregnancy. Running time 63 minutes. New York, 1991, Xenejenex Health Care Communications Companies. $24.95 (videotape).

Perinatal Anesthesia and Critical Care. Edited by James H. Diaz. 395 pages, illustrated. Philadelphia, 1991, W.B. Saunders Company. No price listed.

Physiologie de la Grossesse. Second edition. Edited by Michel Tournaire. 290 pages, illustrated. Paris, 1991, Masson. No price listed.

Preventing Prenatal Harm. Should the State Intervene? Edited by Deborah Mathieu. 153 pages. Boston, 1991, Kluwer Academic Publishers. $45.

Principles and Practice of Medical Genetics. Second edition. Volumes 1 and 2. Edited by Alan H. Emery and David L. Rimoin. 2035 pages. New York, 1991, Churchill Livingstone, Inc. $255.

Principles and Practice of Medical Therapy in Pregnancy. Second edition. Edited by Norbert Gleicher. 1362 pages, illustrated. East Norwalk, Connecticut, 1992, Appleton & Lange. $110.

Reconstructive Surgery in Gynecology. Edited by P.G. Knapstein, V. Friedberg, and B.-U. Sevin. 300 pages, illustrated. New York, 1991, Georg Thieme Verlag. No price listed (hard cover).

Relief From Carpal Tunnel Syndrome and Other Repetitive Motion Disorders. Edited by Norra Tannenhaus. 118 pages. New York, 1991, Dell Publishing. $3.99.

Reproductive Endocrinology. Third edition. Edited by Samuel Yen and Robert Jaffe. 1042 pages,

illustrated. Philadelphia, 1991, W.B. Saunders Company. $135.

Reproductive Risks and Prenatal Diagnosis. Edited by Mark I. Evans. 369 pages, illustrated. East Norwalk, Connecticut, 1992, Appleton & Lange. $85.

Stop Smoking! A Special Program for Pregnant Women. Dissemination packet of eight booklets. 67 pages. Houston, 1991, University of Texas School of Public Health. $75 (donation).

Textbook of Gynecologic Oncology. Edited by G.R.P. Blackledge, J.A. Jordon, and H.M. Shingleton. 512 pages, illustrated. Philadelphia, 1991, W.B. Saunders Company. $120.

Ultrasound in Gynecology and Obstetrics. Textbook and Atlas. Edited by Eberhard Merz, Werner Golhofer, and Ilan E. Timor-Tritsch. 341 pages, illustrated. New York, 1991, Georg Thieme Verlag. No price listed (hard cover).

Ultrastructure of the Ovary. Edited by G. Familiari, S. Makabe, and P.M. Motta. 327 pages, illustrated. Boston, 1991, Kluwer Academic Publishers. $190.

Vaccines for Fertility Regulation. Edited by G.L. Ada and P.D. Griffin. 305 pages. New York, 1991, Cambridge University Press. $20.

60 Ways to Make Stress Work for You. Edited by Andrew S. Slaby. 123 pages. New York, 1991, Bantam Books. $4.50.

What Women Should Know About Chronic Infections and Sexually Transmitted Diseases. Edited by Pamela Patrick Novotny. 150 pages. New York, 1991, Dell Publishing. $3.99.

What You Can Do About Asthma. Edited by Nathaniel Altman. 162 pages. New York, 1991, Dell Publishing. $3.99.

What You Can Do About Epilepsy. Edited by Joan Rachel Goldberg. 147 pages. New York, 1991, Dell Publishing. $3.99.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

4th Annual Review Course in Obstetrics & Gynaecology, May 6-9, 1992, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA Category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

Office Gynecology, April 20-24, 1992, The Hyatt Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, PA/C, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

Origins of Fetal Brain Injury: Part II, May 20-21, 1992, Peoria Civic Center, Peoria, Illinois. Jointly sponsored by the Department of Obstetrics and Gynecology, University of Illinois College of Medicine, at Peoria, and Saint Francis Medical Center. For information contact: The Perinatal Office at Saint Francis Medical Center, 530 N.E. Glen Oak Ave., Peoria, Il 61637. Tel.: (309) 655-2136.

The 2nd International Conference on the Extracellular Matrix of the Reproductive Tract will be held May 20-23, 1992, in Toronto, Ontario, Canada. The program includes plenary sessions, mini symposia, free communication, and posters. For further information regarding program content contact: Phyllis C. Leppert, MD, PhD, University of Rochester School of Medicine and Dentistry. Tel.: (716) 336-3784; fax: (716) 338-3798. For registration information contact: Office of Continuing Professional Education, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Box 677, Rochester, NY 14642-8677. Tel.: (716) 275-4392; fax: (716) 473-1482 (attn: Box 677).

Second Congress of the European Society of Contraception, Astir Palace Complex, "Nafsika" Hotel, Vouliagmemi, Athens, Greece, May 6-9, 1992. Secretariat: c/o G. Creatsas, MD, FACS, 9 Kanari St., Athens 10671, Greece. Tel.: +301-3624367, +301-7770850. Fax: +301-3620484, +301-3229448.

The 3rd Annual Conference on The Prevention of Prematurity and Neonatal Mortality, March 26-28, 1992, at the Westin Peachtree Plaza in Atlanta, Georgia. Sponsored by Emory University School of Medicine, with an educational grant from Healthdyne, Inc., Department of Professional Education. Conference Chair: John Huddleston, MD. For further information contact Dr. McEntire at (800) 366-2229 or Emory Office of Continuing Medical Education at (404) 727-5695.

All Michigan OB/GYN Review Course, May 4-9, 1992, Laurel Manor Banquet and Conference Center, Livonia, Michigan. For further information contact: Division of Continuing Medical Education, Wayne State University School of Medicine, University Health Center, 4201 St. Antoine, 4-H, Detroit, MI 48201. Tel.: (313) 577-1180.

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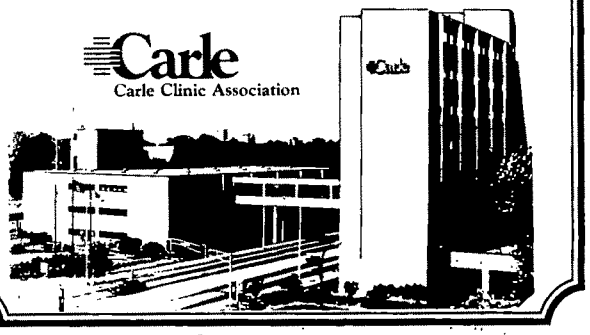
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Please send letter of interest with CV to Jack Jacob, M.D., Alaska Neonatology Associates, P.C., 3340 Providence Drive, Suite 366, Anchorage, Alaska 99508 or call (907) 563-3026.

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OB/GYN Physicians needed to join Carle Clinic Association, a 225 physician, multi-specialty group practice in central Illinois with opportunities at the main campus as well as three additional sites - Urbana, Bloomington, Danville and Mattoon/Charleston. These communities offer recreational, cultural and educational amenities. Benefit package includes a competitive salary with partnership and income sharing plan after two years, professional liability insurance, health, life and disability insurance and generous vacation and meeting time. Contact:

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Medical Director
Carle Clinic Association
602 W. University, Urbana, Illinois 61801
Phone 217-383-3399 - Fax 217-383-3163



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THE AGA KHAN UNIVERSITY

Faculty of Health Sciences
Medical College

The Aga Khan University Hospital is now in its seventh year of operation. The Department of Obstetrics and Gynaecology was commissioned in January 1987. At present there are five full-time faculty members working in the department. The out-patient clinics are busy; patient volume and clinical workload is gradually increasing.

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Candidates applying for this position should be senior academic persons who would be interested in practicing, teaching and training Obstetrics and Gynaecology in a developing university. This post will be suitable for a sabbatical assignment.

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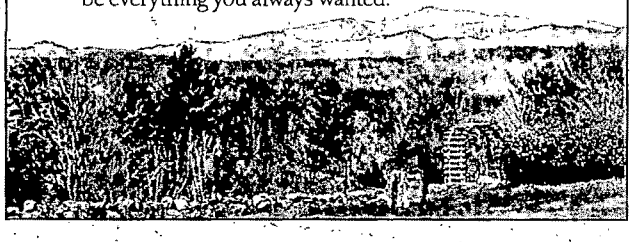
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The Department of OB/GYN at the Columbia Presbyterian Medical Center is seeking a Board Eligible/Certified Physician for GYN coverage in the emergency room five nights per week. Position is available July 1, 1992.

Competitive salary, malpractice included. Apply in writing to: Harold E. Fox, M.D., Vice Chairman, Dept. of OB/GYN, College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032. Columbia University is an equal opportunity employer.

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Salary commensurate with education, length of experience, and academic level. Send resume to Dr. Harold E. Fox, Vice Chairman of Clinical Affairs, Dept. of OB/GYN, Columbia University, 630 West 168th Street, New York, NY 10032. Columbia University is an equal opportunity employer.

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or call (602) 773-2552

CALL FOR ABSTRACTS: Bristol-Myers Squibb/ Mead Johnson Research Symposium 1992

GROWTH FACTORS IN PERINATAL DEVELOPMENT

Hosted By: The Perinatal Research Institute, Children's Hospital Medical Center, University of Cincinnati and Indiana University School of Medicine, Section of Neonatal-Perinatal Medicine

Date: September 10-11, 1992

Location: The Westin Hotel, Cincinnati, Ohio

Program Chairman: Reginald Tsang, M.D., James Lemons, M.D., William Balistreri, M.D.

Registration: Before 6/30/92: $150; After 6/30/92: $175. Checks payable to: The Perinatal Research Institute (in U.S. dollars)

Hotel Reservations Deadline: August 10, 1992; (513) 621-7700 or (800) 228-3000

Abstract Requirements: First author must be a fellow or faculty within 5 years of completion of training. Abstracts will be reviewed. Selected abstracts will be presented. Travel awards available for authors of selected abstracts. Abstracts are not for publication.

For abstract form & information contact: Dr. Reginald Tsang or Ms. Renee McKenzie, The Perinatal Research Institute, Univ of Cincinnati, 231 Bethesda Ave, Cincinnati, OH 45267-0541; (513) 558-1771

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
600 Highland Avenue,
H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
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Faculty Position
Maternal Fetal Medicine

Southern Illinois University School of Medicine
Springfield, Illinois

The Department of Obstetrics and Gynecology at Southern Illinois University School of Medicine, Springfield, Illinois is expanding the Division of Maternal Fetal Medicine and is seeking one new faculty at the Assistant or Associate Professor level. The division serves as a state-of-the-art perinatal referral center for central and downstate Illinois and is responsible for providing tertiary care to high risk obstetrical patients, including antenatal testing, perinatal ultrasonography (including Doppler), and fetal evaluation and therapy with planned expansion of its prenatal diagnostic services to include CVS. Division faculty are also responsible for the development of educational curriculum and delivery of instruction to medical students, residents, and fellows. The division is committed to research activities pertinent to maternal fetal medicine, and research opportunities are available. Certification or eligibility in the subspecialty is required.

Send Curriculum Vitae to: Robert D. Hilgers, M.D., Professor & Chairman, Department of OB/GYN, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794. Phone: (217) 782-8247. Or send inquiries to Robert C. Kaufmann, M.D., Chief, Division of Maternal Fetal Medicine. Phone: (217) 785-5540

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Maternal Fetal Medicine Fellowship Department of Obstetrics and Gynecology

Southern Illinois University School of Medicine
Springfield, Illinois

The Department of Obstetrics and Gynecology at Southern Illinois University School of Medicine, Springfield, Illinois has one first year Fellowship position available in Maternal Fetal Medicine beginning July 1, 1993. This is a two year accredited Fellowship program in which both clinical and research training are emphasized. Clinical material is drawn from the 3000 deliveries/year at St. John's Hospital Perinatal Center where fellows participate in a supervised, in-depth experience in management and treatment of medical and obstetrical complications with emphasis on antenatal diagnostic procedures and ultrasound techniques. This flexible program incorporates a minimum six month period of full time research and can accommodate various clinical/research objectives based on the fellow's individual goals. Fellows are encouraged to present papers and to represent the Maternal Fetal Medicine Division at national scientific meetings.

Board certified or eligible candidates should send their Curriculum Vitae to:
Robert C. Kaufmann, M.D., Department of OB/GYN
Southern Illinois University School of Medicine
P. O. Box 19230, Springfield, IL 62794
Phone: (217) 782-8240

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One full-time position is available in the Department of Obstetrics and Gynecology, University of California, Davis, Medical Center, Sacramento, as Director of the Division of Maternal/Fetal Medicine with concurrent appointments to the faculty of the University of California, Davis, School of Medicine. Applicants for this perinatologist position should be Board eligible or certified in Obstetrics and Gynecology and must have successfully completed subspecialty training in Maternal/Fetal Medicine with subsequent Specialty Board eligibility and/or certification in Maternal Fetal Medicine. Applicants must possess or be eligible for medical licensure in the State of California. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities or potential for such activities. Applicants must demonstrate experience in prenatal diagnosis, ultrasound and genetics.

Responsibilities of this position include direction and responsibility for research activities in the division, both inpatient and outpatient case management, surgery and procedures specific to high risk obstetrics, participation in teaching activities with residents, interns and medical students, development and/or participation in research activities and involvement in University and Hospital committees. Serves as Administrative Director of the Division.

Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
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UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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The Department of Obstetrics, Gynecology and Reproductive Sciences of the University of California, San Francisco and Planned Parenthood San Francisco/Alameda are seeking a full time joint position for a Board Eligible or Board Certified Obstetrician/Gynecologist with an interest in ambulatory reproductive health care. The position includes half time medical directorship at Planned Parenthood with responsibilities for administering and monitoring medical standards in contraception, abortion, sterilization and infertility.

This position includes a faculty appointment and academic activities in the Department of Obstetrics, Gynecology and Reproductive Sciences at the University of California. Salary and academic rank are negotiable depending on qualifications and experience.

Please send curriculum vitae to:

Philip Darney, M.D.
Department of Obstetrics and Gynecology
San Francisco General Hospital
1001 Potrero Avenue
San Francisco, California 94110

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Perinatologist

The Department of Obstetrics and Gynecology of the **University of Wisconsin-Madison** seeks a board certified **Perinatologist** to direct its Section of Maternal-Fetal Medicine. In addition to patient care and resident and student teaching, essential duties will include acting as liaison with referring physicians and other hospitals in the region. An active research interest in some aspect of perinatal medicine is also necessary. Faculty rank (Associate Professor or Professor) is dependent on experience. Send CV and application to:

Chester B. Martin, Jr., M.D.
University of Wisconsin, Madison
H4/654 Clinical Science Center
600 Highland Avenue
Madison, WI 53792

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ASSISTANT/ASSOCIATE PROFESSOR MATERNAL-FETAL MEDICINE

The University of North Carolina at Chapel Hill Department of Obstetrics and Gynecology in conjunction with the Wake Medical Center, Raleigh, North Carolina, and the Wake Area Health Education Center has an opening for a board certified or active candidate perinatologist with a special interest in high risk pregnancies and ultrasound. The position is in the Wake AHEC OB/GYN Department which has three full-time obstetricians and gynecologists, plus the program director who is board certified in Maternal-Fetal Medicine. Wake Medical Center is located in Raleigh, approximately 40 minutes from Chapel Hill, and is a 560-bed comprehensive tertiary care center with over 4000 deliveries per year and a neonatal intensive care unit staffed by four neonatologists. Excellent opportunity for research and teaching residents and medical students rotating through Wake Medical Center at all times. Collaboration with the Maternal-Fetal Medicine Division at Chapel Hill is encouraged and anticipated. Competitive salary and excellent fringe benefits.

Write or contact:

Juan L. Granados, M.D.
OB/GYN Wake AHEC Director
Wake Medical Center
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The Department of Obstetrics and Gynecology at the University of Utah School of Medicine is seeking a faculty member for the General Obstetrics and Gynecology Division at the Instructor or Assistant Professor level. Qualification include Board Certification or an active candidates for certification by the American Board of Obstetrics and Gynecology, demonstrated ability and interest in teaching, research and patient care. Interest in routine obstetrics. If interested, please send curriculum vitae and bibliography to:

James R. Scott, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
University of Utah School of Medicine
50 North Medical Drive
Salt Lake City, Utah 84132

Applications must be postmarked by May 1, 1992. Applications received after that deadline may be considered if no qualified applications have been received by that time. The University of Utah is an Equal Opportunity Affirmative Action Employer.

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted. Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiform, Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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Artist's enhancement of scanning electron microscopy: *C albicans* 24 hours posttreatment with butoconazole nitrate.*

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1. Droegemueller W, Adamson DG, Brown D, et al. Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol.* 1984;64:530-534. *Micrographs on file, Syntex Laboratories, Inc. †In pregnant patients (second and third trimesters only), therapy should be extended to 6 days. Please see adjacent page for brief summary of prescribing information. © 1991 Syntex Laboratories, Inc. 813-12-196-91

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PALO ALTO, CA 94304

Committed to the OB/GYN and women's healthcare

FEMSTAT® PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

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Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

ENHANCING YOUR PERCEPTION OF ORAL CONTRACEPTION

*Serious as well as minor side effects have been reported with use of all oral contraceptives. The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

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TRI·LEVLEN® Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen

TRI-LEVLEN® 28 TRI-LEVLEN® 21

BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's–but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

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INDICATIONS AND USAGE
TREATMENT

CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Proteus mirabilis,* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D. pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K. pneumoniae*), *E. coli, Proteus mirabilis,* and *Serratia marcescens**.

Skin and Skin Structure Infections due to *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus* species (excluding enterococci), *Escherichia coli, Klebsiella pneumoniae, Peptococcus niger**, *Peptostreptococcus* species.

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *Streptococcus agalactiae, E. coli, Proteus mirabilis, Neisseria gonorrhoeae,* Bacteroides species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus niger* and *Peptostreptococcus* species).

Cefotetan, like other cephalosporins, has no activity against *Chlamydia trachomatis.* Therefore, when cephalosporins are used in the treatment of pelvic inflammatory disease, and *C. trachomatis* is one of the suspected pathogens, appropriate antichlamydial coverage should be added.

Intra-abdominal Infections caused by *E. coli, Klebsiella* species (including *K. pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B. distasonis, B. ovatus, B. thetaiotaomicron*) and *Clostridium* species*.

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic *in vitro* against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

BEFORE THERAPY WITH CEFOTAN IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOTETAN DISODIUM, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. IF THIS PRODUCT IS TO BE GIVEN TO PENICILLIN-SENSITIVE PATIENTS, CAUTION SHOULD BE EXERCISED BECAUSE CROSS-HYPERSENSITIVITY AMONG BETA-LACTAM ANTIBIOTICS HAS BEEN CLEARLY DOCUMENTED AND MAY OCCUR IN UP TO 10% OF PATIENTS WITH A HISTORY OF PENICILLIN ALLERGY. IF AN ALLERGIC REACTION TO CEFOTAN OCCURS, DISCONTINUE THE DRUG. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE TREATMENT WITH EPINEPHRINE AND OTHER EMERGENCY MEASURES, INCLUDING OXYGEN, INTRAVENOUS FLUIDS, INTRAVENOUS ANTIHISTAMINES, CORTICOSTEROIDS, PRESSOR AMINES AND AIRWAY MANAGEMENT, AS CLINICALLY INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including cefotetan, and may range from mild to life-threatening. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is a primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to discontinuation of the drug alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *Clostridium difficile.*

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: The administration of CEFOTAN may result in a false-positive reaction for glucose in the urine using Clinitest®†, Benedict's solution, or Fehling's solution. It is recommended that glucose tests based on enzymatic glucose oxidase be used.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Pregnancy: Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy. Those appearing in italics have been reported during postmarketing experience.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700); *pseudomembranous colitis.* Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment or surgical prophylaxis. (See WARNINGS.)

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300); *agranulocytosis, hemolytic anemia, leukopenia, thrombocytopenia,* and *prolonged prothrombin time.*

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700); *anaphylactic reactions.*

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

Miscellaneous: *Fever*

In addition to the adverse reactions listed above which have been observed in patients treated with cefotetan, the following adverse reactions and altered laboratory tests have been reported for cephalosporin-class antibiotics: urticaria, pruritis, Stevens-Johnson syndrome, erythema multiforme, toxic epidermal necrolysis, vomiting, abdominal pain, colitis, superinfection, vaginitis including vaginal candidiasis, renal dysfunction, toxic nephropathy, hepatic dysfunction including cholestasis, aplastic anemia, hemorrhage, increased BUN, increased creatinine, elevated bilirubin, pancytopenia, and neutropenia.

Several cephalosporins have been implicated in triggering seizures, particularly in patients with renal impairment when the dosage was not reduced (see DOSAGE AND ADMINISTRATION and OVERDOSAGE). If seizures associated with drug therapy occur, the drug should be discontinued. Anticonvulsant therapy can be given if clinically indicated.

DOSAGE AND ADMINISTRATION
TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Skin & Skin Structure		
Mild-Moderate[a]	2 grams	2 g every 24 hours IV 1 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6 grams[b]	3 g every 12 hours IV

[a] *K. pneumoniae* skin and skin structure infections should be treated with 1 or 2 grams every 12 hours IV or IM.

[b] Maximum daily dosage should not exceed 6 grams.

If *C. trachomatis* is a suspected pathogen in gynecologic infections, appropriate antichlamydial coverage should be added, since cefotetan has no activity against this organism.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. See FULL PRESCRIBING INFORMATION for a complete description.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

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Bethesda, Maryland, Los Angeles, California, Boston, Massachusetts, Galveston, Texas, Chicago, Illinois, and Toronto, Ontario, Canada

Optimal fetal outcome in hyperphenylalaninemic pregnancies appears to occur when maternal blood phenylalanine levels <10 mg/dl are achieved by 8 to 10 weeks' gestation and maintained throughout pregnancy.

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John E. Buster, MD, Peter R. Casson, MD, Arthur B. Straughn, PharmD, Debra Dale, RN, Edward S. Umstot, MS, Neil Chiamori, PhD, and Guy E. Abraham, MD

Memphis, Tennessee, and Santa Monica and Torrance, California

Single doses of oral micronized dehydroepiandrosterone were absorbed efficiently and converted to dehydroepiandrosterone sulfate and testosterone, but not estradiol, in postmenopausal women.

Endothelium-derived relaxing factor and indomethacin-sensitive contracting factor alter arterial contractile responses to thromboxane during pregnancy 1171

Carl P. Weiner, MD, Loren P. Thompson, PhD, Kang-Zhu Liu, MD, and James E. Herrig, BS

Iowa City, Iowa

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Lp(a) lipoprotein: Relationship to cardiovascular disease risk factors, exercise, and estrogen 1182

Rogerio A. Lobo, MD, Morris Notelovitz, MD, PhD, Leslie Bernstein, PhD, Farouk Y. Khan, MD, Ronald K. Ross, MD, and Wellington L. Paul, PhD

Los Angeles, California, and Gainesville, Florida

Lp(a) lipoprotein levels were higher in patients with cardiovascular disease but were not influenced by age, sex, or exercise; oral estrogen had a minor effect.

Lipid and apolipoprotein abnormalities in hirsute women. I. The association with insulin resistance 1191

Robert A. Wild, MD, Petar Alaupovic, PhD, and Ivy J. Parker, BS, RN

Oklahoma City, Oklahoma

Gonadotropin-releasing hormone analog was used to remove gonadal sex steroid influence on lipoprotein metabolism in hirsute women to dissect out the independent effects of insulin resistance.

Elevated maternal plasma corticotropin-releasing hormone levels in pregnancies complicated by preterm labor 1198

Wendy B. Warren, MD, Sharon L. Patrick, MD, and Robin S. Goland, MD

New York, New York

Maternal plasma levels of placental corticotropin-releasing hormone are elevated in pregnancies complicated by preterm labor; these elevations may reflect early activation of the placenta in mechanisms leading to preterm labor.

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New York, New York, Santiago, Chile, Santo Domingo, Dominican Republic, Los Angeles and San Francisco, California, Singapore, Jyväskylä, Finland, and Uppsala, Sweden

After contraception with levonorgestrel implants or with levonorgestrel-releasing or copper-releasing intrauterine contraceptive devices, pregnancy rates and outcomes were within normal limits.

Progression of diabetic retinopathy in pregnancy: Association with hypertension in pregnancy 1214

Barak Rosenn, MD, Menachem Miodovnik, MD, Jane Khoury, MS, George Kranias, MD, C. Andrew Combs, MD, PhD, Francis Mimouni, MD, Tariq A. Siddiqi, MD, and Matthew J. Lipman, MD

Cincinnati, Ohio

Progression of retinopathy during pregnancy in insulin-dependent diabetes is associated with glycemic control in early pregnancy, chronic hypertension, and pregnancy-induced hypertension.

Prenatal ultrasonographic findings associated with wide clefting of the upper two thirds of the fetal sternum 1219

Paula R. Mahone, MD, David M. Sherer, MD, Carl D'Angio, MD, and James R. Woods, Jr., MD

Rochester, New York

We describe the first report of prenatal ultrasonographic findings associated with wide clefting of the superior two thirds of the fetal sternum.

The presence of platelet-activating factor binding sites in human myometrium and their role in uterine contraction 1222

Yan-ping Zhu, PhD, R. Ann Word, MD, and John M. Johnston, PhD

Dallas, Texas

Platelet-activating factor receptors are present in human myometrium; this autacoid causes myometrial contraction, a change in intracellular Ca^{2+}, and myosin light-chain phosphorylation.

Effects of platelet-activating factor on prostaglandin E_2 production by intact fetal membranes 1228

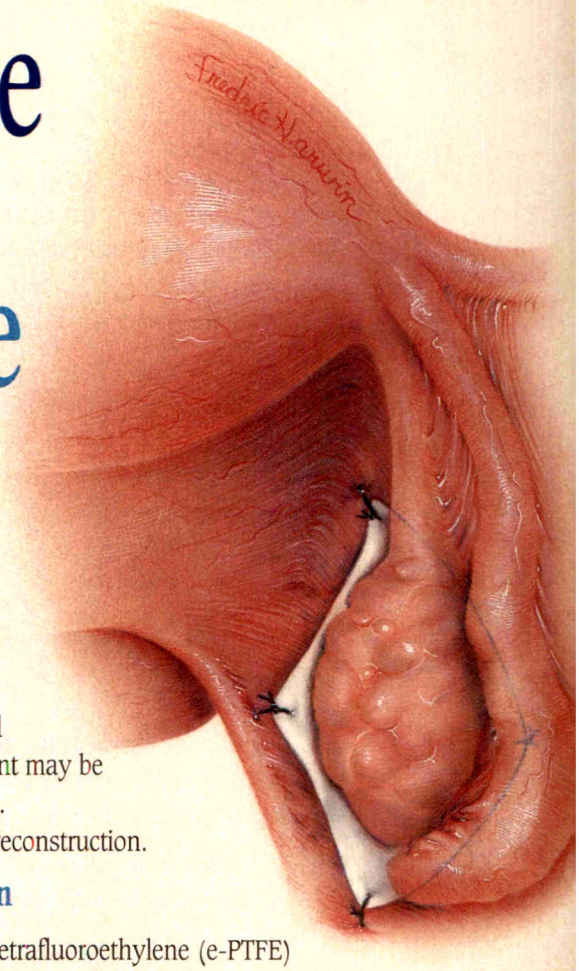
Christine Morris, MB, BS, Haroon Khan, MD, Mark H.F. Sullivan, PhD, and M.G. Elder, MD

London, England

Platelet-activating factor mainly stimulated prostaglandin E_2 production by the chorion-decidual component of human fetal membranes and markedly increased prostaglandin E_2 production after labor.

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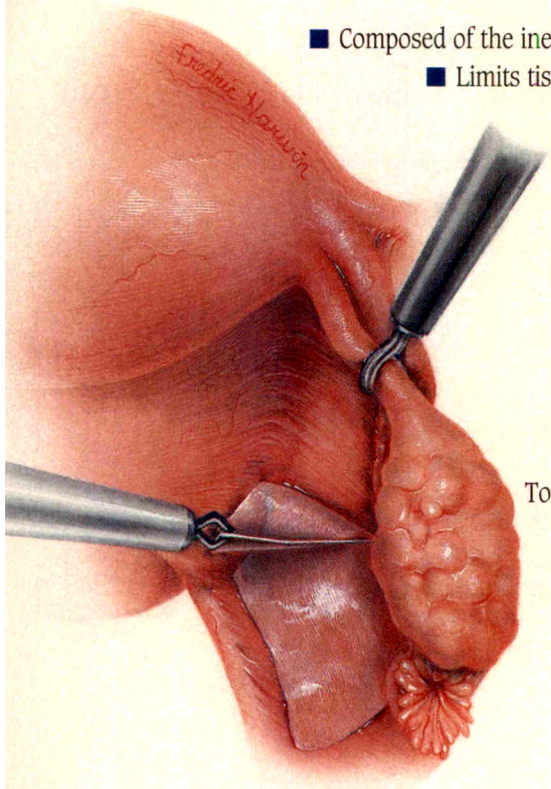


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New York and Brooklyn, New York, and Chicago, Illinois

High rates of cytologic abnormality and an association between abnormality and severity of immunodeficiency occurred in 135 human immunodeficiency virus-infected women from three ambulatory care sites.

Intravenous nitroglycerin for uterine relaxation of an inverted uterus 1237
Kenneth M. Altabef, MD, James T. Spencer, MD, and Stanley Zinberg, MD
New York, New York

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Severe anti-C hemolytic disease of the newborn 1239
John M. Bowman, MD, Janet M. Pollock, RT, Frank A. Manning, MD, and Chris R. Harman, MD
Winnipeg, Manitoba, Canada

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Andry Vleeming, PhD, H. Muzaffer Buyruk, MD, Rob Stoeckart, PhD, Sacid Karamursel, MD, and Chris J. Snijders, PhD
Rotterdam, The Netherlands, and Istanbul, Turkey

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Miguel Zamora-Garza, MD, Joel Rizo, MD, and Alberto Dominguez, MD
Mexico City, Mexico

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Masaharu Ito, MD, Toshimitsu Nakamura, MD, Toshihiro Yoshimura, MD, Hideki Koyama, MD, and Hitoshi Okamura, MD
Kumamoto, Japan

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J.L. Benedet, MD, G.H. Anderson, MB, and J.P. Matisic, MD

Vancouver, British Columbia, Canada

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Andrew J. Satin, MD, Kenneth J. Leveno, MD, M. Lynne Sherman, RN, and Donald D. McIntire, PhD

Dallas, Texas

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Middle cerebral artery flow velocity waveforms in normal and small-for-gestational-age fetuses 1262

Giancarlo Mari, MD, and Russell L. Deter, MD

New Haven, Connecticut, and Houston, Texas

The pulsatility index of the fetal middle cerebral artery has a parabolic pattern during pregnancy; pulsatility index below the normal range is frequently associated with an adverse perinatal outcome.

Inferior vena cava flow velocity waveforms in appropriate- and small-for-gestational-age fetuses 1271

Giuseppe Rizzo, MD, Domenico Arduini, MD, and Carlo Romanini, MD

Rome and Ancona, Italy

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Diathermy loop excision in the management of cervical intraepithelial neoplasia: Diagnosis and treatment in one procedure 1281

Karel G.G. Keijser, MD, Peter Kenemans, MD, Petronella H.Th.H. van der Zanden, Charles P.T. Schijf, MD, G. Peter Vooijs, MD, and Rune Rolland, MD

Nijmegen and Amsterdam, The Netherlands

Long-term follow-up demonstrates that diathermy loop excision of the whole transformation zone is an efficient combined diagnostic and therapeutic procedure in patients with cervical intraepithelial neoplasia.

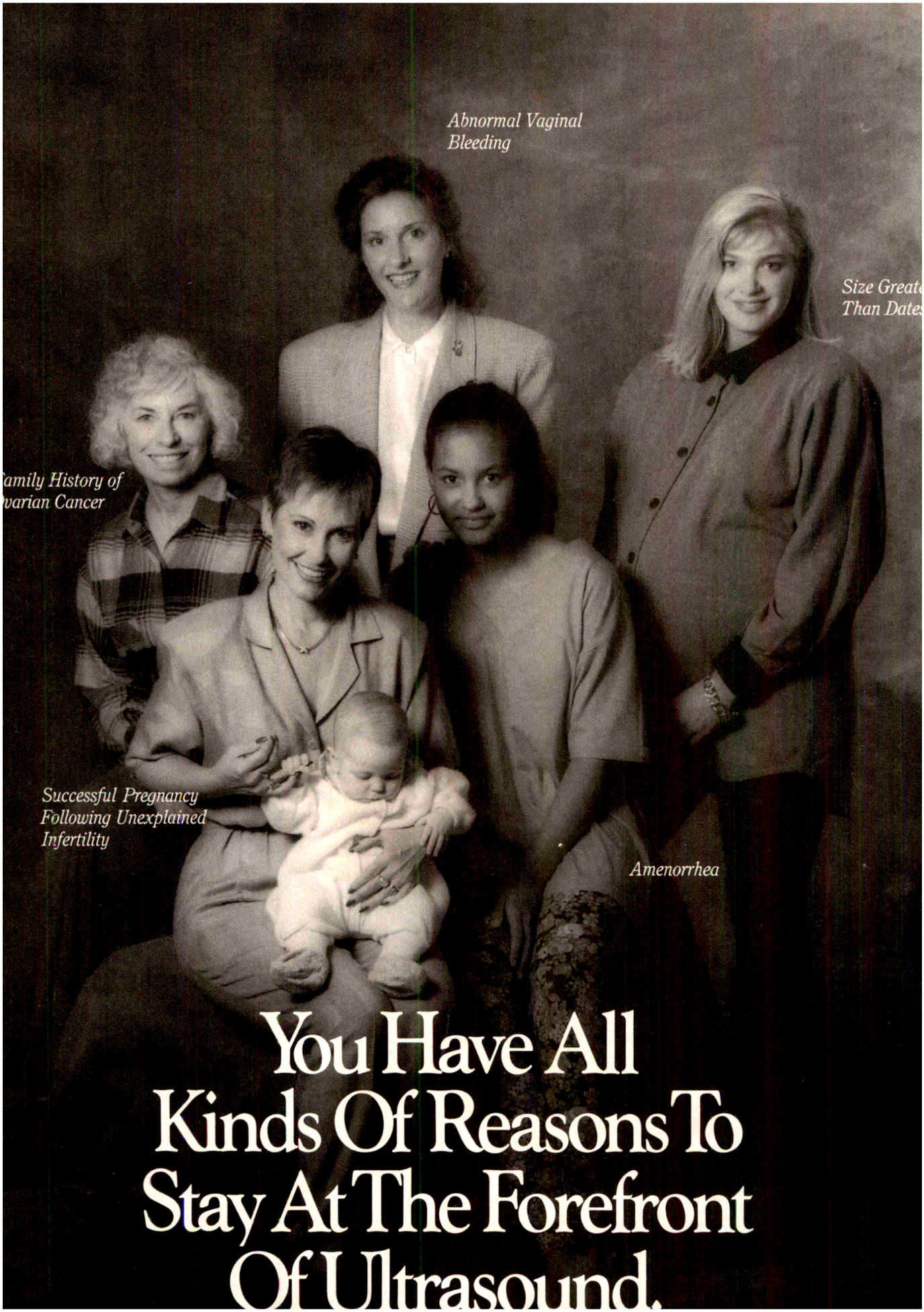
Fetal polycythemia and thrombocytopenia in pregnancies complicated by maternal diabetes mellitus 1287

Douglas R. Salvesen, MD, Michael J. Brudenell, MD, and Kypros H. Nicolaides, MD

London, England

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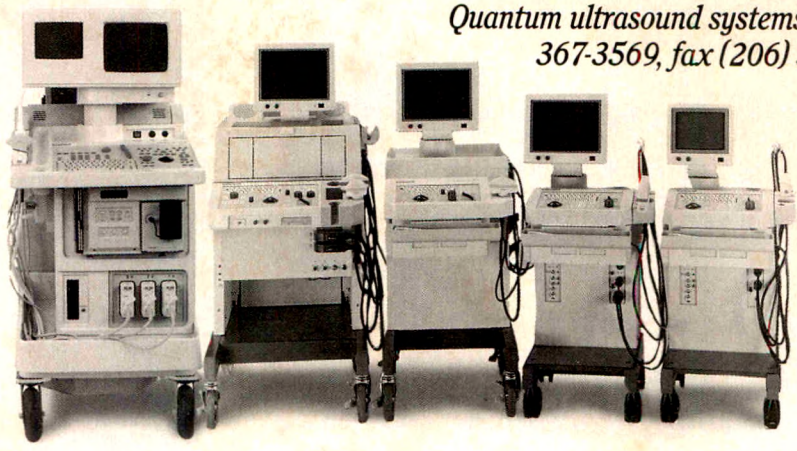
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Jim van Eyck, PhD, and Birgit Arabin, PhD
Zwolle, The Netherlands, and Berlin, Germany

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Phyllis August, MD, Beth Marcaccio, MD, Joseph M. Gertner, MD, Maurice L. Druzin, MD, Lawrence M. Resnick, MD, and John H. Laragh, MD
New York, New York

Serum levels of 1,25-dihydroxyvitamin D are lower at the time of delivery in women with preeclampsia compared with normotensive and chronically hypertensive pregnant women.

Long-term sequelae of acute pelvic inflammatory disease: A retrospective cohort study 1300
Sharon Safrin, MD, Julius Schachter, PhD, Donna Dahrouge, MPH, and Richard L. Sweet, MD
San Francisco, California

At a median of 37 months after acute pelvic inflammatory disease, frequency of infertility was 40%, chronic pelvic pain 24%, and subsequent pelvic inflammatory disease 43%.

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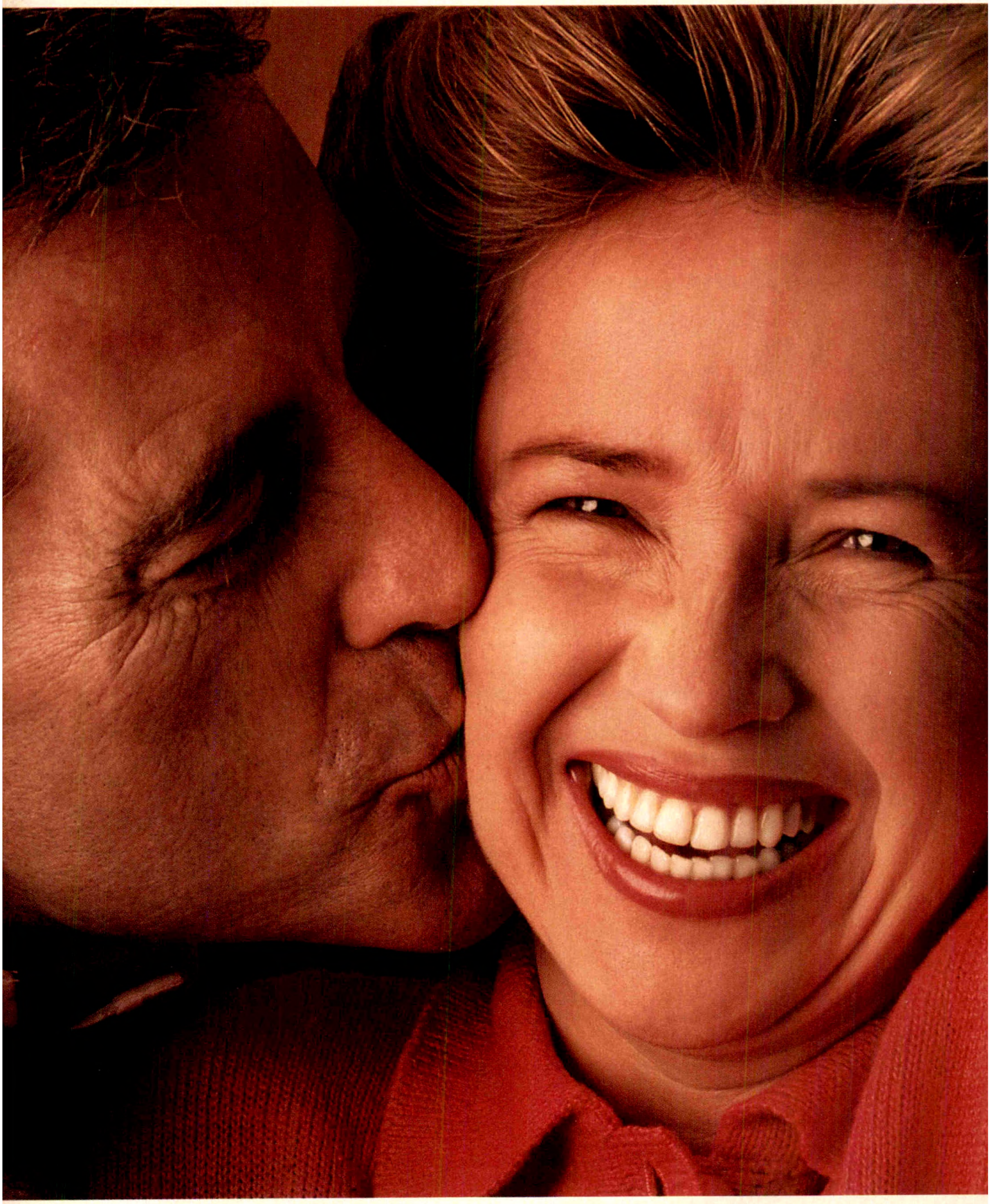
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2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
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WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risks, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

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2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy: changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

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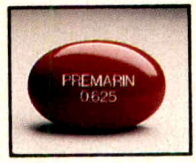
1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.
Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.
Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream
Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.
The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.
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Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.
Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

Reference: 1. Weiss NS, Ure CL, Ballard JH, et al: Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. *N Engl J Med* 1980;303:1195-1198.

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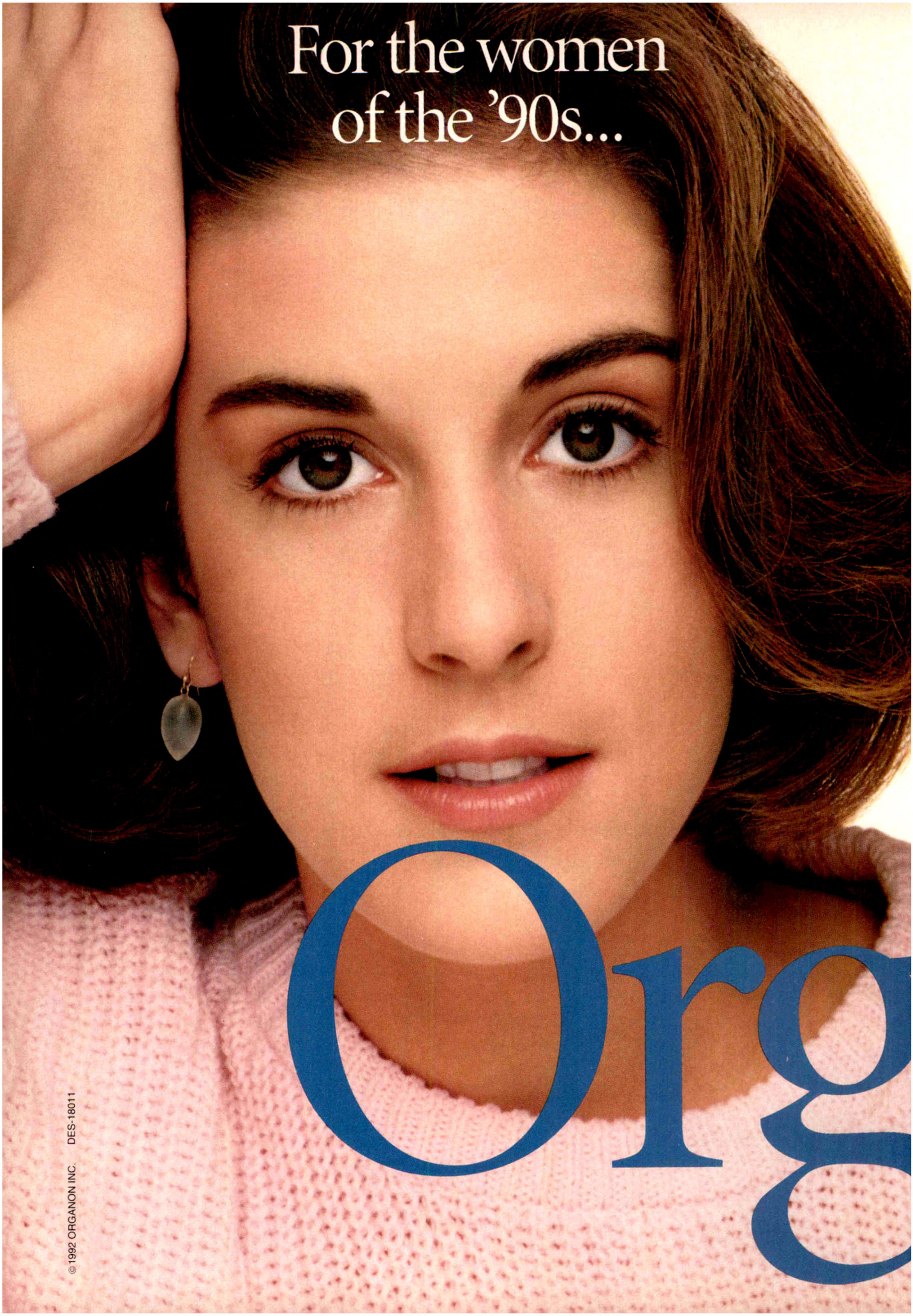
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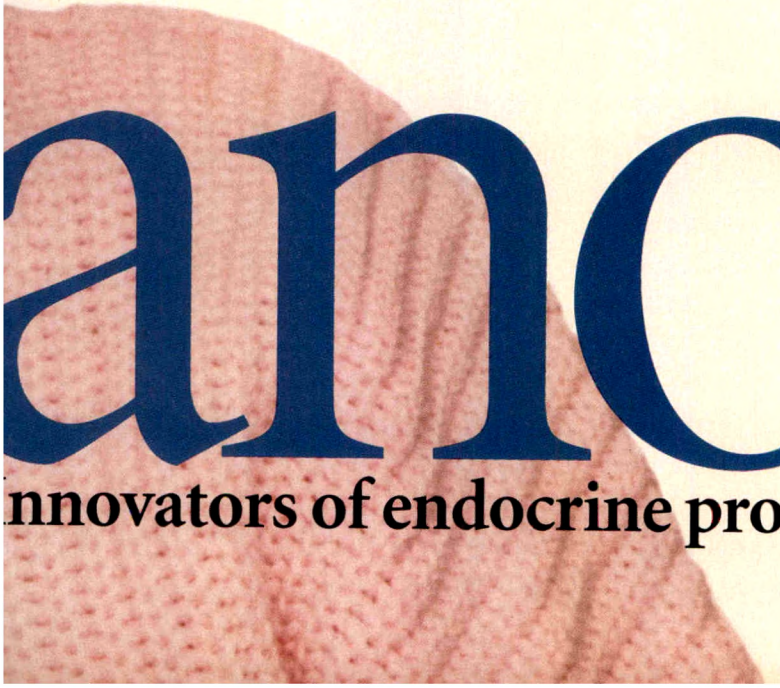
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Manuscript submission. Manuscripts should be submitted to one of the two Editors in Chief as follows:

1. **Dr. Zuspan**—manuscripts from the eastern and southeastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. J. Peter Van Dorsten, P.O. Box 34, MCV Station, Richmond, VA 23298.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

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Abstract page including key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Below the abstract, list 3 to 5 *key words or short phrases* for indexing purposes.

A structured abstract is required for certain types of articles and a *standard abstract format* is required for other types of articles; therefore the author(s) should select and prepare the appropriate abstract to be used in the submitted manuscript. This applies to both independently submitted and society manuscripts.

Structured abstract. A structured abstract, limited to 150 words, is to be used for regular articles, society articles, and Current Investigation articles; excluded are Clinical Opinion, Current Development, case report, and brief communication articles. The structured abstract is to contain the following major headings: *Objective(s); Study Design; Results;* and *Conclusion(s).* The *Objective(s)* reflects the purpose of the study, that is, the hypothesis that is being tested. The *Study Design* should include the setting for the study, the subjects (number and type), the treatment or intervention, and the type of statistical analysis. The *Results* include the outcome of the study and statistical significance if appropriate. The *Conclusion(s)* states the significance of the results.

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Text. Do not hesitate to write your manuscript in the first person and active voice if they are more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the

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With the exception of Current Development articles, the length of text material (introduction through *Comment* section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

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Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

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In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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Abortion or motherhood, suicide and madness

Kenneth J. Ryan, MD

Boston, Massachusetts

This is the first presidential address for this society in three years. Because of personal or family illness, the last two presidents unfortunately had no opportunity to exercise this distinct honor and privilege. I count myself lucky therefore to be able to stand before you, in what is for me a life-affirming event, and renew the tradition of the presidential address at a meeting of The American Gynecological and Obstetrical Society.

I have chosen neither a safe nor an easy subject for this presentation, but one which is instead provocative and morally contentious. The title is "Abortion or Motherhood, Suicide and Madness." I have chosen this title to highlight the political turmoil abortion engenders. When Joseph Califano, Secretary of Health, Education, and Welfare under the Carter Presidency, asked a special group to come up with alternatives to abortion, which he and Carter opposed, the report concluded that the only real alternatives are "suicide, motherhood and some would add, madness."[1]

Abortion is a worldwide problem. In the 1970s, there were twice as many abortions as births in the Soviet Union. In France births equaled abortions, while in England, with very liberal abortion laws, there were six times as many births as abortions. In Brazil, Italy, Japan, Portugal, Spain, Sweden, and Yugoslavia the average was 1.5 to 3 births for every termination. In many of these countries abortions are illegal and the numbers are estimates. In most cases the number of abortions seemed to be connected to the availability of sex education, contraceptives, and birth control programs rather than laws.[2]

From the Department of Obstetrics, Gynecology, and Reproductive Biology, Harvard Medical School.

Presidential Address presented at the Tenth Annual Meeting of the American Gynecological and Obstetrical Society, Carlsbad, California, September 5-7, 1991.

Reprint requests: Kenneth J. Ryan, MD, Department of Obstetrics, Gynecology, and Reproductive Biology, Harvard Medical School, 75 Francis St., Boston, MA 02115.

6/6/35921

Indeed, the place of abortion in our society is crying out for a fair and rational yet civilized discussion. I will try to give you one. The frustration of dealing with the topic of abortion today was well summed up by Anna Quindlen in a *New York Times* book review of Laurence Tribe's *Abortion, the Clash of Absolutes.* Ms. Quindlen wrote: "The last time I was on a panel on the subject of abortion, the adrenaline flowed like wine and I made a solemn vow: that I would never again engage in public debate on the subject. It was not simply that it was difficult, and bitter, and personal, and nasty. It was that it led nowhere. At the end of almost three hours, none of us had budged from our original positions. There was heat, but no light. It was a 'clash of absolutes.'"[3] It has not always been so difficult to have an unemotional discussion on abortion. The change to implacable, polarized positions on the subject followed in the aftermath of the Supreme Court decision on *Roe v Wade* in 1973.[4] We will review what led up to and followed this momentous and unexpected change in abortion laws in the United States.

On Jan. 22, 1973, the decision of the Supreme Court not only declared the criminal abortion laws of Texas and Georgia unconstitutional but mandated some revision in abortion statutes for almost every other state of the Union. It was a remarkable decision, passed by a vote of 7 to 2 with three of Nixon's four court appointees not only siding with the majority but one of them (Blackmun) delivering the majority opinion. Only John F. Kennedy appointee Byron White and Nixon appointee William Rehnquist voted in the minority. I remember that Monday distinctly since I had just arrived at Harvard and the Boston Lying-in Hospital to take up a new post 3 weeks earlier. The hospital public relations office called and insisted that I give an immediate press conference so they could deal with a barrage of calls from the news media. There was considerable interest in Massachusetts about what the change in law meant in practical terms since almost all

local women seeking an abortion up to this time were forced to travel to New York, which had repealed its antiabortion statutes in 1970. The reporters all wanted to know when that could stop. At the conference I welcomed the Supreme Court decision to make abortion largely a matter between a woman and her physician and hoped that there would be enough medical support in Massachusetts so that patients would not have to travel for care. My short vignette on all the television channels was followed by Archbishop (later Cardinal) Humberto Medeiros of Boston asserting that abortion was nothing less than murder. It was a chilling reception to the new politics of abortion.

Some background of the Supreme Court case provided by Laurence Tribe[5] is fascinating. The Roe of *Roe v Wade* was a pseudonym for Norma McCorvey who explained that, at age 25 and unmarried, she was gang raped when walking home from work in 1969. Abortion was illegal in Texas except to save a woman's life and she claimed she couldn't afford to travel to obtain a legal abortion elsewhere. Although that pregnancy was no longer at issue by the time the appeal reached the Supreme Court, the case was still heard since no case concerning pregnancy could otherwise reach settlement, and the justices reasoned that the plaintiff might even be pregnant and have a similar problem in the future. Ironically McCorvey admitted 15 or so years later that she hadn't been raped but made up the story to hide the fact that she'd gotten pregnant in a more usual manner. The Wade of *Roe V Wade* was a Dallas county prosecutor who is sued regularly whenever a Texas law is challenged.

A companion complaint was heard at the same time: *Mary Doe* (a pseudonym) *v Bolton* (Attorney General of Georgia). Mary Doe was really Sandra Race Cano, a 22-year-old married Georgia citizen who was 9 weeks pregnant with three living children. Two of the oldest children were placed in a foster home because Mary Doe was too poor and couldn't care for them. The youngest child was placed for adoption. Her husband had abandoned her and she was living with her indigent parents and their eight children. She had been a mental patient. Although she became reconciled with her husband, he was a construction worker who had only sporadic jobs. She was advised that having an early abortion would be less dangerous to her than carrying the pregnancy to term. The abortion committee of the Grady Memorial Hospital denied her request as not complying with the Georgia law. The Georgia statute was actually more enlightened than that of Texas. In Georgia an abortion was possible on grounds of threats to the woman's life or health, rape, or if the fetus would be born with serious defects. The procedure had to be performed in an accredited hospital and approved by a staff abortion committee, and the decision had to be concurred with by two additional physicians. Mary Doe eventually had the child, Melissa Able, who is now herself a mother. Ironically, today Mary Doe is active in the pro-life movement and her daughter (who obviously wouldn't have been born if aborted in 1969) is pro-choice.[4,5] Norma McCorvey, the original Roe, is still pro-choice.

The chief architect of the *Roe v Wade* decision was Harry Blackmun, who finally filled the seat vacated by Abe Fortas in 1970 after two prior Nixon nominees, Judges Haynsworth and Carswell, had failed the approval process. Blackmun was considered a moderate conservative but neither Nixon nor his attorney general, Mitchell, knew his judicial philosophy. The "litmus" test applied to prospective court nominees for beliefs on abortion hadn't yet gained currency. Nixon reportedly at least wanted to be assured that Blackmun's three daughters in their 20s were not "hippie types." They weren't. It was a happy coincidence that Blackmun had been general counsel to the Mayo Clinic for 10 years, which undoubtedly prepared him for dealing with the medical aspects of his report.[6]

Justice Blackmun, writing for the Supreme Court, concluded that a woman's right to terminate a pregnancy was so fundamental that government could not interfere with the exercise of that right except for compelling reasons. In the first trimester the government could not interfere except to insist that a physician perform the abortion. In the second trimester regulation might involve only procedures to protect the woman's health. In the third trimester, after viability was achieved, the state could protect fetal life and prohibit abortions unless the life or health of the woman was threatened. Blackmun reviewed the history of abortion from Greek and Roman times, through the English common law and nineteenth-century American state laws. He recognized the controversy about the Hippocratic oath and the Pythagorean origins for the injunction against abortion. In common law, abortion was not considered a crime before quickening, and whether it was felony or misdemeanor after quickening is in dispute. Later abortion laws were supposedly designed to protect the health of the woman and to require that only physicians do a "legal" abortion.[4]

Support in the Constitution was sought by the justices in the liberty clause of the Fourteenth Amendment as a right of privacy in reproductive matters and in the unenumerated rights reserved for the people in the Ninth Amendment. This was the basis for two prior court decisions on the use of contraceptives if married (*Griswold v Connecticut,* 1965) and if unmarried (*Eisenstadt v Baird,* 1972). In deciding whether Roe was decided correctly, I rely on Laurence Tribe, a constitutional lawyer who has prevailed in some 16 of 20 appearances before the Supreme Court. Tribe points out

that our protection by the Bill of Rights does not apply to actions of state governments but specifies only what Congress cannot do. It is the Fourteenth Amendment, enacted in 1868, that prohibits the states from making or enforcing laws that abridge our rights as United States citizens and prohibits the states from depriving us of life, liberty, or property without due process of law. This liberty clause applies the Bill of Rights to the states, although according to Tribe some provisions of the Bill of Rights are still not protected in all states. Judge Robert Bork, whose nomination to the Supreme Court failed in 1987, has been an outspoken critic of *Roe v Wade*. His feeling is that the court should not enumerate rights not written in the constitution and the liberty clause of the Fourteenth Amendment could be abridged by adequate "due process of law." Although not specified in the Constitution, the right to privacy was invoked prior to the Griswold and Baird decisions in the Supreme Court rulings in 1923 *(Meyer v Nebraska)*, which allowed parents to teach their children German over state objections due to the war, and in 1925 *(Pierce v Society of Sisters)*, which overruled state laws that would have shut down parochial schools. In 1967, the Court struck down state law against interracial marriage *(Loving v Virginia)*. In *Skinner v Oklahoma* (1942) the court recognized the right of reproduction by striking down a sterilization law for criminals. The Supreme Court has also invalidated criminal convictions based on evidence obtained from stomach pumping and from surgical removal of a bullet from a suspect. Clearly some right to privacy must be implicit in these prior findings. Tribe quotes Judge Bork as saying: "The right to abort, whatever one thinks of it, is not to be found in the Constitution." Tribe responds that neither are such words as freedom of marital choice, freedom of parenthood, and family self-determination and, one might add, freedom to use contraceptives. The privacy and liberty issues are broader than the question of abortion and will undoubtedly be played out in future court decisions. Whether the Roe decision was illegitimate judicial activism depends on whether one sides with Robert Bork or Laurence Tribe.[5] One conservative justice, Lewis Powell, appointed by Nixon in 1971 had a somewhat different prospective. His father-in-law was an obstetrician in Richmond, Virginia, and he had two brothers-in-law who were obstetricians. He had "heard the stories of unsanitary butchers and coat-hanger abortions." He felt the right-to-privacy argument was tenuous and based his rationale instead on the opinion of the federal judge who struck down the Connecticut abortion law, viz: "The moral positions on abortion about which each side was so sure must remain a personal judgment, one that people may follow in their personal lives and seek to persuade others to follow, but a judgement they may not impose upon others by force of law."[6] To test the strength of this opinion, we might look at what led up to and has followed the *Roe v Wade* decision.

The pre-Roe period

Condit, in her book on rhetoric and abortion, points out that between 1870 and 1950 up to 30,000 abortions were done "legally" each year in the nation's hospitals. Many more illegal abortions were done outside of hospitals under less than sanitary and safe conditions. In the 1950s and 1960s the number of illegal abortions that showed up in the hospitals as incomplete or septic abortions numbered in the hundreds of thousands. The locus of abortion shifted and by 1971, prior to Roe, up to 600,000 legal abortions were being done each year within hospitals.[7] Many of us had professional if not personal experiences with these events. I went to Harvard Medical School in the late 1940s, when disseminating information on contraception or fitting a diaphragm was illegal in the state of Massachusetts. I learned little about family planning or abortion in those days. I was in residency training in medicine at Columbia-Presbyterian Hospital in New York, and I vividly remember the young woman who died of acute tubular necrosis after a "coat hanger" procedure, steadfastly denying to the end that she had sought an abortion. I shudder a little at the memory of the coroner's post-mortem examination downtown at the Bellevue Hospital morgue. Stories like these, of the death and disability of women from illegal abortion, were beginning to have a public impact.

It took some major events in the 1960s to really engage the general public interest. Sherri Finkbine was a happily married mother of four and the host of the children's television program Romper Room in Phoenix, Arizona. She was pregnant for the fifth time and unwittingly used a tranquilizer picked up by her husband in Europe. It was thalidomide and the gross deformities caused by the drug were by then well known. Ordinarily, she might have had an abortion in her hospital on the grounds of a "psychiatric crisis," but the publicity she created by warning other women created an uproar. The hospital was threatened with suits by religious groups since the Arizona law only allowed abortion to save the woman's life. Sherri Finkbine fled to Sweden where, as Ms. Condit describes: "A deformed fetus was scraped from the womb of this 'all American' woman." This tragic case challenged the prevailing ideas of who needed abortions and why and openly challenged the legal system. Mrs. Finkbine loved children and worked with them. In fact she had a normal child 2 years later. During this same period amniocentesis and prenatal diagnosis became clinical realities and consideration of abortion for fetal indications was born.[7]

The other major event of the 1960s was the rubella epidemic of 1964 and 1965 chronicled in an edition of *Life* magazine. I was a new and young professor and Chairman of Obstetrics and Gynecology at MacDonald House and Western Reserve University in Cleveland. Any prior ideas I had about abortion were lost in the deluge of demands for pregnancy terminations for rubella-induced fetal deformity. The requests were coming from all segments of society and all religious persuasions. Some of my colleagues tried to refer patients from Mississippi and the Dakotas. Although the law in Ohio forbade abortions except to protect the woman's life, at that time I told the hospital trustees that we could no longer use the ploy of a threatened suicide and a psychiatric certification for the abortion. The public debate on abortion was in a sense officially opened. The American Law Institute, composed of academicians and practicing attorneys from the major cities, was circulating its 1959 proposed revision of the model penal code. They suggested the now widely accepted "hard" justifications for abortion: to protect the woman's life or when pregnancy would gravely affect her physical or mental health or when the child would be born with defects or was the result of rape or incest. They recommended that two other doctors provide certification.[5] This proposal was ahead of its time, but no one could expect that the Supreme Court would in a little over one decade go beyond these conservative guidelines.

The events of the 1960s prompted the convening of an International Conference on Abortion in Washington, D.C., in 1967, organized by Eunice and Sargent Shriver and sponsored by the Kennedy Foundation and the Harvard Divinity School. All the "important" constitutional lawyers and sociologists and ethicists and physicians were there. Father Robert Drinan, the future congressman but then Dean of Boston College Law School, felt it would be better to have no laws on abortion like those promulgated by the American Law Institute than to sanction the procedure by law. The ethics debate centered around the balance of values between the interests of the pregnant woman and those of the fetus. In asserting the humanity of the fetus, Paul Ramsey, the conservative Protestant theologian, believed the fetal soul enters the "body" at conception but observed that an abortifacient pill would make abortion a "private" matter. John Noonan presented his history of the attitude of the Catholic Church to abortion, which varied depending on whether quickening was considered the time of ensoulment and even whether the time varied between male and female fetuses. Later he would write that the Church was always against abortion. I wrote: "Even though a pregnancy may not be life threatening, it may be life-devastating enough to force women to desperately seek and obtain abortion by any means possible even at considerable risk to their life. Living with children in poverty, sustaining a pattern of futility of life, living with a hopelessly deformed or retarded child or bearing an illegitimate one without paternal support is a life situation many women will not accept. This is a medical and social fact, moral, legal, and religious issues notwithstanding." I also noted that abortion only interrupts an acute episode of what may be a more chronic or recurrent problem, be it social behavior, poverty, or mental illness. Other forms of therapy are needed to prevent unwanted pregnancy by exercise of personal responsibility and use of contraception. The report of this conference was never published but rather condensed, slanted away from a strong pro-reform consensus, and popularized in the form of a paperback book with a pro-life preface by Pearl Buck.[8] Still and all, this was a civilized conference on abortion in which the discussants appeared to be communicating with one another, but no new ground was broken.

I have always maintained that the reason the ethical debate about abortion is so intractable is because we lack a compelling analogy with other moral conflicts for which reasonable solutions have been devised. The fetus is in and of the pregnant woman. How can society force her to use her body against her will for purposes of gestation when there is no other, even close example of such a requirement for men or for women in other circumstances even to save a life. An apt negative analogy of this is the failure of society to force a father or mother to donate an organ for a needy child. In most instances pregnancy is a greater burden than a bone marrow transplant is. In point of fact, the law does not require that dead persons yield up their organs to save a life without permission. "A Defense of Abortion,"[9] written by Massachusetts Institute of Technology philosopher Judith Jarvis Thomson in 1971, tried to conjure up the imagery of an analogy to abortion. You have been chosen by the Society of Music Lovers because of a compatible blood type to be abducted, drugged, and forcibly connected to the circulatory system of a famous violinist who is dying of kidney disease so that his system can be dialyzed. You wake up and are told that it's unfortunate that this has happened to you but if you unplug yourself the poor violinist will die. This is a balancing act between your right to use your body as you see fit and another's right to life. Your plight poses the question of whether you are morally obligated to carry such a burden and at least raises the symmetry of cases in which a woman is pregnant as the result of being raped. Since the Thomson argument might justify abortion for a viable fetus and doesn't address the issue of when a woman voluntarily puts herself at risk of pregnancy, it fails to carry the day. Abortion politics continued to reflect this conflict be-

tween a woman's choice and the fetus' right to life, but in the post-Roe era the pro-life and pro-choice movements plummeted headlong into two opposing, entrenched camps of ideology.

The post-Roe era

After the *Roe v Wade* decision there were many pro-life-sponsored attempts, most unsuccessful, in the Congress and in the state legislatures to limit access to abortions in various ways. In 1976 the Supreme Court quickly rejected a Missouri statute requiring spousal consent for a first-trimester abortion. Other state statutes including imposing criminal liabilities on physicians to ensure special duties to the possibly viable fetus, limitation on abortion techniques such as saline infusion, restriction of where procedures could be performed to hospitals only, zoning exclusions of abortion clinics, mandating the presence of two physicians in late abortions, the need to provide physical descriptions of fetal development to the pregnant woman, and the requirement for pathologic tissue analysis in all abortions were all ultimately declared unconstitutional. In *Bellotti v Baird* the Supreme Court struck down the requirement for parental consent for minor women. On the other hand, in *Maher v Roe,* the Supreme Court ruled that Connecticut did not need to fund a nontherapeutic abortion with public funds. In 1980, with the *Harris v McRae* decision, the Hyde Bill, which allowed use of Medicaid funds for abortion only if the mother's life was in jeopardy or when the pregnancy was the result of rape or incest, was declared constitutional. Although women had a constitutional right to abortion, the government did not have to fund them. In these two judgments, the foes of abortion scored their first major successes. Constitutional amendments had also been proposed in order to apply personhood to the unborn from conception but never generated sufficient momentum. In 1981, Senator Helms' Human Life Bill defining persons as existing from conception for the purposes of constitutional protection was defeated. In 1983, the proposed Hatch amendment asserting that "A right to abortion is not secured by the Constitution" was also defeated by the Senate.[5]

Ronald Reagan, fortieth President of the United States, may leave his mark in history for his impact on the abortion question. He appointed three conservative justices to the Supreme Court, Sandra Day O'Connor, Antonin Scalia, and Anthony Kennedy, replacing three who voted for the *Roe v Wade* decision, and elevated a Roe opponent, William Rehnquist, to Chief Justice. Reagan openly encouraged and supported a human life amendment and legislative efforts to discourage abortions. The centerpiece of this effort was his 1984 essay, "Abortion and the Conscience of the Nation."[10] Reagan made the comparison of the Dred Scott decision of 1857, which took citizenship away from blacks, with the Roe decision, which denied "citizenship" to fetuses. He cast abortion as the moral equivalent of slavery and referred to the book, *Abortion: The Silent Holocaust.* In the introduction to the book containing Reagan's essay, it was noted, as Condit observed, "none too bashfully" that his writing "is a historic document if only because it evokes the moral passion of Abraham Lincoln against slavery."[7] This was part of a pro-life strategy to motivate the activists and win over the religious and "traditional" Americans. Even the lessons of history were cast in a pro-life mode by John Noonan's book, *An Almost Absolute Value in History.* This was an effort to make the antiabortion tradition not only Catholic and Christian but secular as well. In addition to casting abortion as slavery, as holocaust, and as a consistent wrong through all history, the icons were mobilized. These visual symbols included pictures of fetuses, dismembered fetuses, the tiny feet of a 10-week fetus, and concentration camp victims side by side with fetal corpses. The movie *The Silent Scream,* containing a sonogram of an abortion in progress, was widely distributed. A fertilized egg, a blastocyst, an embryo, and a fetus were always described as an unborn baby. The rhetorical effect was potent and powerful and undoubtedly led to more activism and even violence.[7] Abortion clinics were invaded, vandalized, and bombed, and the number of incidents reached more than 100 in 1984.[11, 12] Relatively peaceful demonstrations before the Supreme Court on the Roe anniversaries were replaced by a religious war in Operation Rescue. Operation Rescue, which is unabashedly religious in its rhetoric and ardor, organized pro-life volunteers nationally to disrupt the operations of abortion clinics. Its leaders come from the evangelical Christian sects but it also has Catholic representation.[13] Unfortunately the politicians and the Justice Department have done more to encourage than to dissuade this sometimes violent form of "civil" disobedience. In the invasion of Witchita, Kansas, by Operation Rescue, to disrupt and close down abortion clinics in August 1991, the state governor addressed the Operation Rescue group at a rally. The United States Attorney General sided with them in a challenge to an injunction against blocking clinic access by Federal Judge Patrick Kelly. Judge Kelly even received personal death threats.[14] Pro-life activists do engender death threats to those opposing them, even though their leaders may try to distance themselves from this form of intimidation.

In 1989, the Supreme Court, with the three new Reagan appointees concurring, upheld sharp state limits on abortion for the first time. The case, *Webster v Reproductive Health Services* involved Missouri state laws that banned abortions from public hospitals and banned public employees from performing them unless

the woman's life was at risk. The law further required tests for viability if the fetus was at least 20 weeks' gestation. In the preamble of the Missouri law, it was asserted that life begins at conception, but the Court did not address this. Blackmun, in dissent, noted the religious rather than secular basis for stating that life begins at conception and felt it violated the Establishment Clause of the First Amendment separating church and state.[15] The extreme interest in this case was evidenced by the fact that more amicus curiae briefs were filed for Webster than in any prior Supreme Court case.[5] The assault on Roe had begun in earnest, and many felt this was an invitation for the states to enact laws that could be used to test complete reversal. This was also an invitation for political activism on this issue at the state legislative level. There was a sense that we were returning to one-issue politics.

Further partisanship by the federal executive branch under President Bush is reflected in the Department of Health and Human Services "gag rule," which limits what providers in federally funded Title X contraceptive clinics can say about abortion, which is nothing, except that it is not a form of family planning. In the 1991 *Rust v Sullivan* decision the Supreme Court upheld this as constitutional.[16] This intrusion of government into control of speech by a physician to a patient should bother all physicians regardless of how they feel about abortion.

The public and the polls

Public opinion polls have been used extensively since the Roe decision. The problem, according to sociologists, is that polls may make rather than mirror public opinion; they always slant questions and oversimplify the diversity of society. In any case, during the past 15 years some results are consistent and unchanging. Some 20% of Americans oppose abortion on essentially all grounds, 20% approve abortions without restriction, and the majority approve abortions under limited and specified conditions. The "hard" justifications for abortion—protecting life, physical health, severe deformity of the fetus, and rape and incest—generally have a substantial majority supporting them.[17] While most Americans do not want to make abortion illegal, sizable numbers respond yes when asked if abortion is murder.[18] There is also a greater willingness to countenance early rather than late abortions. The major arguments are against abortion on demand, as birth control, or for convenience, sex selection, or social reasons. Some see hope of compromise around the selective limitation of abortion, but the activists on both sides have shown little "give." Mary Ann Glendon[19] of Harvard Law School has suggested we should be more like the French, have restrictive abortion laws and then get around them in some way.

Kristen Luker has studied the activists and found the pro-life group spending more time at it; they are more apt to be blue collar, married housewife-mothers, high school graduates, Catholic or religious, and male professionals. The pro-choice group are more apt to be white collar, unmarried professional or working women, often without children, college graduates, and not regularly attending church.[20] Often personal experiences are the motivation for activism. Kate Michelman, Executive Director of the National Abortion Rights Action League, tells the story of expecting her fourth child when her husband, who had fallen in love with another woman, asked for a divorce. When Michelman requested an abortion, the hospital panel told her she could have the procedure if the husband who had just abandoned her would give his consent. Not surprisingly, Ms. Michelman thinks women themselves ought to make this decision.[5, 21] There is the story of Becky Bell who was afraid to tell her parents about her pregnancy, took a home abortifacient, and died. Her father became an activist to quash consent laws that require parental notification.[3]

Ethics and religion

In the United States in the late 1980s half of all pregnancies are unintended (3.4 million) and roughly half of these are aborted (1.6 million). More than half of the unintended pregnancies occur in women who don't use contraception, which dramatically reflects the potential impact of family planning education on the abortion numbers. Those seeking abortion are generally under age 25, unmarried, economically deprived, and either in school, working, or both.[22] In the book *Pregnant by Mistake* by Maxtone-Graham,[23] women with unwanted pregnancies were interviewed. They generally break down into three categories. The first group of women used birth control responsibly, knew beforehand that they would seek an abortion if contraceptives failed, and did not act out of emotion or impulse at the time of the unwanted pregnancy. These women were acting in a "moral" way according to the reviewer, and even if someone disagreed with their rationale, one would be hard put to challenge their moral intentions. Another set of women were self-characterized as being psychologically "messed up" with traumatic personal histories to match. The reviewer felt that berating them for immorality would be a futile exercise. A third group was described as young, naive, and incapable of dealing with the world in a morally functional way.

Those women who could discuss their moral principles felt that using contraceptives, being consistent in their attitude about unwanted pregnancy, and considering the interest of others constituted a rational moral defense for their actions. Some felt that having a child should require more justification than having an abortion. They did not want to bear a child in the absence of a loving, stable environment. Bearing a child and

then giving it up for adoption was considered by many women to be cruel. Some women bore their first child and gave it up for adoption because they didn't know of the abortion option. In a second pregnancy they chose abortion. As one woman expressed it, "Carrying a pregnancy to term is different from abortion, those months of carrying the child were very destructive to me. That was the end of my trust in people." Most of these women resented the male domination of the abortion debate and the limits drawn up by the law, medicine, and the church, with insufficient input from women. They also resented the implication that if you voluntarily have sex, you must accept the responsibility for bearing and raising any child that ensues even it it is a failure of contraception. These women looked on sex as an inevitability of life, like eating. While each act of sex, like each meal, might be voluntary, eating and having sex over a lifetime are not voluntary in any practical sense of the word.[7]

What I have ascertained in these women's experiences is a form of descriptive ethics that is all too often neglected by the ethicists, who tell people how to live their lives from the perspective of applied or normative ethics without knowing what those lives are really like. A good deal of the ethical debate has centered on the right of the fetus to life having greater moral force than any woman's need, save direct threats to her life or physical health. The pro-life support for life, a human being, a person, or whatever to exist at conception is, in my view, an attempt to secularize what is largely a religious issue, the sanctity of the reproductive act and anything that ensues from it. There is an attempt thereby to deny any morally relevant differences between a fertilized egg, a preembryo, an embryo, a fetus, and a newborn infant at term and to morally trump any but the strictest justifications for abortion. I will grant that after a stage of development that would allow viability outside the womb, one is hard-pressed to find morally relevant differences between the advanced-stage fetus and the newborn, but there are such differences. These differences diminish as term approaches but never disappear; witness the caution with which the law now deals with treatment decisions that might violate the pregnant woman's wishes. That caution is engendered by the fact that the fetus is inside the woman's body. In this conflict between the supposed absolute right of the fetus to be born and the woman to use her body as she chooses, one must recognize that fetal life is contingent life. It is contingent on normal development, which is by no means certain in nature (there is a 60% attrition rate), and it is contingent on invading and using the woman's body for its development. Those contingencies are the morally relevant differences. The right of a woman to the use of her body as she chooses cannot be overcome in early pregnancy by any secular argument that contingent fetal life has some superior claim. If one invokes souls and the sanctity of contingent fetal life, one can make an argument in favor of the fetus that is religiously grounded and morally defensible but not in a pluralistic democracy. Stronger arguments of a secular nature can be made for limiting abortions after viability is reached, but for my part I do not want to defend late abortions at all. After viability, one of the contingencies of fetal life is removed, and if the pregnancy must be terminated, it can be with the expectation of treating the fetus as a premature newborn when delivered. Independent life begins at birth; there is no secular or scientific justification for believing otherwise. Equating contingent fetal life with independence is a rhetorical device that contributes to lack of respect due the moral views of those who disagree and foments the kind of religious fervor seen in Wichita in the hot summer of 1991.

Not all religious views hold that abortion is always wrong or that life begins with conception. There is no mention of abortion in the Bible. One passage in Exodus 21:22, 23 (NAS) stipulates that "If men struggle with each other and strike a woman with child so that she has a miscarriage, yet there is no further injury, he shall be fined as the woman's husband may demand of him; and he shall pay as the judges decide. But if there is any further injury than you shall appoint as penalty life for life." Clearly the woman's life carries greater weight than the miscarriage. Note also that the husband (not the woman) sets the fine in the male tradition that miscarriage deprived the father of an heir.

I want to close with some quotes from a sermon on abortion given by a minister in a Southern Baptist Church in Waco, Texas.[24] It is the most eloquent and wise reading I have seen on the subject.

"Thousands of years of debate . . . and still there is no consensus about when human life becomes a human person. Despite the efforts of movies like *The Silent Scream,* no scientific information can yet determine when unborn life becomes a human person. Just as medical science has never absolutely, clearly and without an ounce of reservation been able to determine when a dying person loses personhood, science cannot tell us with absolute clarity when personhood begins.

"But neither has the church formed a consensus on the question . . . . One of the reasons for this lack of consensus is that the Bible has no clear word. I repeat, there is no place in the Bible where it said: 'Thou shalt not have an abortion.'

"The primary Jewish position is that human personhood begins when the baby is born and draws breath. Genesis 2:7 declares that 'God breathed into his nostrils the breath of life and man became a living soul.' Elsewhere there is a place in Old Testament law *(noted above)* . . . .

"The Roman Catholic Church has held its very clear position only since 1869 and Pope Pius IX. It has always been against the abortion of a human being . . . .

"Protestant groups have taken a whole range of positions . . . . Thus we must invoke the Protestant principle which says that in cases where Scripture is not clear, final interpretation should be left to each individual person, given guidance within his or her community of faith.

"We must be very careful in even mentioning God's will. We cannot glibly claim God's will is involved in every conception, I cannot ascribe to God's will conception by rape or by incest or by a couple in no position to marry and parent a child. These are all occurrences in a tragic and sinful world. Thus, abortion is not the automatic breaking of God's will. Neither can we say that it is God's will that we perform abortion . . .. He understands—even allows—and certainly forgives the choices we have to make.

"And the Church's role? It is to guide in the decision-making process by doing what I'm doing: safeguarding the woman's freedom of religion to make the choice; to affirm her in her willingness to bear a child under difficult circumstances if that is her choice; and if she decides instead to have an abortion, not to tell her what she should be feeling. Instead:

> if she feels guilt, to offer and act out the Grace of God
>
> if she feels grief, to offer and act out the Comfort of God
>
> if she feels remorse, to offer and act out the Peace of God
>
> if she feels fear, to offer and act out the Love of God . . . .

"Since there is no moral consensus or biblical mandate we should accord women the legal, moral, and religious freedom to choose.

"This sermon is an interim report, influenced by the thinking of others, and struggled with at a personal level by one who does not have all the answers. It is the best I can do, for now. I say it with apprehension and some sadness. It is as clear as I can think, and no clearer—it is as simple as I can speak, but no simpler. May God have grace and understanding for women who must make such decisions—and for preachers who talk about it."[1, 24]*

To this, I can only add amen! I included the material on ethics and religion in this last section to try to make the point that there is more than one morally and religiously respectable position on abortion. We should vigorously oppose the moral self-righteousness of any extreme position for or against abortion and help fashion a public policy that does justice to our democratic principles.

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Contraceptive prevalence, reproductive health, and international morality

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William Faulkner said that the past is never dead; it is not even past. On the 18th of September, 1888, the first paper at the first meeting of the American Association of Obstetricians and Gynecologists was delivered by Joseph Price, in Philadelphia, Pennsylvania. The paper was entitled "Drainage in Abdominal Surgery."

In 1927, Professor James Kennedy, a student, an admirer and a successor of Joseph Price, established the Joseph Price Oration with a visionary move to enhance communication with scientists from abroad. The first Joseph Price Oration was given in 1928 by Professor Alfred Scott Warthin, Ann Arbor, Michigan, on "Sex Differences in the Pathologic Picture of Syphilis." I will deliver the fifty-eighth Joseph Price Oration, and the occasion fills me with pride and humbleness.

I have read with admiration the curriculum vitae of Drs. Price and Kennedy. Had I been asked to provide a common epitaph for both of them, I would have selected the words of François de Sales: "*Le bruit ne fait pas de bien, le bien ne fait pas de bruit.*" In English this would mean that the noise doesn't do any good, the good doesn't make any noise. I would like to pay tribute to the memory of two giants in the history of American obstetrics and gynecology.

There is an Oriental proverb saying that when a man is young, he writes songs; grown up, he speaks in proverbs; in old age, he preaches pessimism; and I would like to emphasize that I am not a pessimist. I am not an optimist either; I am simply confused. Why? Because every day I am confronted with three levels of reality: the scientific, the economic, and the political. When those three levels converge, progress can be amazingly rapid. When they don't, which is frequently the case, the political reality assumes a controlling power. This is particularly true in the fields of population, fertility regulation, and reproductive health.

It is generally accepted that at the time Jesus Christ was born the world population was <300 million. It took some 1500 years to double the global population, which occurred around the time of the second voyage of Columbus to America beginning in the sixteenth century. The first billion was reached at the beginning of the nineteenth century amid the turbulence of the Napoleonic wars, and the second billion was reached in 1927 at the very time James Kennedy established the Joseph Price Oration. But now, in <50 years, the population doubled to 4 billion in 1974. The fifth billion was reached in 1987 and the sixth billion is projected to be reached in 1997 or 1998.[1] How did developing country governments react to this development? With concern, as indicated by the fact that in the early 1960s only seven governments provided family planning programs. In the early 1980s more than 120 governments supported such programs.[2] The great increase in the number of governments supporting family planning was accompanied by an impressive rise in contraceptive prevalence worldwide, which was greatly facilitated by breakthroughs in contraceptive technology principally in the United States, exemplified by the development of oral contraceptives and modern intrauterine contraceptive devices. In the early 1960s the contraceptive prevalence in all developing countries was only 9%. In the early 1980s it was 45%.[1]

The initial rationale of developing country governments for supporting family planning programs was undoubtedly the demographic concern. However, the rationale was considerably broadened when those governments recognized that the effective use of family planning would delay the age at first pregnancy and space further births. When integrated into primary health care, both factors will decrease infant, child, and maternal morbidity and mortality and significantly reduce illegal abortions and their health hazards. Hence today it is generally recognized by developing country governments that family planning, maternal care, infant and child care, and the control of sexually transmitted diseases constitute the four fundamental pillars of reproductive health policy.

The United Nations Population Fund estimates that in 1987 the number of contraceptive users worldwide was around 380 million. This corresponds to a contraceptive prevalence of 50% to 51%, which is equivalent

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Table I. Contraceptive method used

Method	*Developing countries (%)*	*Developed countries (%)*
Sterilization	45.3	14.2
Intrauterine contraceptive devices	23.1	7.8
Hormonal	15.5	18.4
Natural	8.5	30.5
Barrier	6.6	21.3
Other	0.9	7.8

Source: United Nations Population Fund, 1990.[3]

to a statement that another 380 million or so couples were not using any form of fertility regulation. In terms of the various methods female sterilization was the method in 119 million users, male sterilization in 45 million, intrauterine contraceptive devices in 84 million, steroidal contraceptives in 67 million, and conventional methods (barrier, periodic abstinence, withdrawal, etc.) were used by 65 million couples.[3] There are enormous regional, national, cultural, and social differences in the contraceptive methods used.[4]

The marked differences hidden beyond these figures are shown by the percentile distribution of various methods in developing and developed countries (Table I). Furthermore, marked regional differences exist; in 1983 contraceptive prevalence was 74% in East Asia, 53% in Latin America, but only 14% in Africa. Female and male sterilization and intrauterine contraceptive devices together accounted for 65% of the contraception in East Asia but for only 3% in Africa.[1]

Another important aspect of the problem is unmet contraceptive need, defined as the number of women of fertile age who do not wish to have more children. It is estimated by the United Nations that more than one third of the 140 million women in the developing world who became pregnant in 1988 did not want to have another child.[5]

A major step in the evolution of ideas in the field was the recognition by an increasing number of developing country governments that the demographic perspective of unmet contraceptive need can best be influenced by addressing the user perspective. The user perspective is dictated by three significant precepts: human rights, reproductive health, and family economy. These precepts are inextricably intertwined with the status and development of women. This powerful conceptual development is reflected by the political policy position of developing country governments. In 1980, 52 governments stated that they supported family planning for the demographic rationale, but 65 said that they provide support because of the human rights and reproductive health rationale, whereas 35 governments still did not provide any support.[6]

What is reproductive health, then, and how is it quantitated? The constitution of the World Health Organization in 1948 defined "health" as a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Viewed against this broad and very positive definition, reproductive health has a number of basic elements such as the *ability* to procreate, regulate fertility, and enjoy sex; *success* in the outcome of pregnancy and in infant and child survival, growth, and development; and *safety* in pregnancy, childbirth, fertility regulation, and sex.[4] It should be emphasized that in all resolutions of the World Health Assembly (the parliament of the World Health Organization [WHO]) the term fertility regulation also includes the prevention and management of infertility.

Reproductive health is assessed by considering various indicators shown in Table II. It should be stated that the figures in Table II represent only the tip of the iceberg of preventable human suffering.

There is an epigram by Stephen Crane[8]:

A man said to the universe:
"Sir, I exist."
"However," replied the universe, "The fact has not created in me
A sense of obligation."

The question is, has it created a sense of obligation in us? WHO reminds us that 85% of the world's births, 95% of the world's infant deaths, and 99% of the world's maternal deaths take place in developing countries.[9] Indeed, the lifetime risk of dying from pregnancy-related causes is 1 in 1750 in developed countries, 1 in 90 in Latin America, 1 in 38 in South Asia, and 1 in 24 in Africa.[10] The WHO estimates that between now and the end of the century 7 million women will die in childbirth. The majority of maternal deaths in developing countries are preventable through prenatal care, basic health care, and family planning at an estimated cost of a mere $2 per capita per year.[11]

Among the major child-killing diseases in developing countries, diarrhea is responsible for 5 million deaths annually, followed by acute respiratory disease, 4 million; measles, 2 million; tetanus, 1.8 million; and malaria, 1 million. One of the underlying factors is malnutrition. The United Nations estimates that the percentage of malnourished children worldwide is 39%; it is 52% in Southeast Asia, 35% in Africa and in the eastern Mediterranean region, and 21% in Latin America.[5]

The annual total of sexually transmitted infections is 250 million new cases each year, according to the recent estimates of WHO.[11] These figures are shown in Table III. It should be mentioned that hepatitis B can also be transmitted sexually, at least in certain endemic areas.

With respect to human immunodeficiency virus, WHO estimates that since the start of the pandemic >1.5 million cases of acquired immunodeficiency syndrome may have occurred worldwide, about 400,000

of which have been pediatric acquired immunodeficiency syndrome cases resulting from perinatal transmission. On the basis of current projections, WHO has estimated that by the year 2000 there may be a cumulative total of 25 to 30 million human immunodeficiency virus–infected adults and 10 million infected infants and children in the world. It is also estimated that during the 1990s about 10 million uninfected children will be orphaned because of their mother's death from acquired immunodeficiency syndrome.[11]

From the data shown here, it is easy to define our goals in reproductive health. It will be much more difficult to realize them. The goals are to reduce the unmet need for family planning, to increase family planning services and methods, to lessen maternal infant and child mortality and morbidity, and to reduce the prevalence of sexually transmitted diseases.

All this will require money, money, and money. As George Bernard Shaw said, "Money is indeed the most important thing in the world, and all sound and successful personal and national morality should have this fact for its basis." (George Bernard Shaw: *The Irrational Knot*). I would like to go a step further and suggest that this must also be the basis of international morality, as suggested by the title of this oration.

Dr. H. Nakajima, the Director General of WHO, recently said that in the developed world annual per capita health expenditure averages $400, whereas in the poorest countries and the least developed countries it is <$5. The missing ingredient, says Dr. H. Nakajima, is the will to help. If we could increase health spending in the developing countries by only $2 per capita, we could immunize all their children, eradicate polio, and provide the drugs to cure all the cases of diarrheal disease, acute respiratory infection, tuberculosis, malaria, schistosomiasis, and sexually transmitted diseases. I may add to this that between 1980 and 2000 the total population of the developing world is expected to increase by 95%, whereas the elderly population (aged ≥65) will probably rise by almost 240%.[12] This will result in an enormous rise in health costs, actualizing the questions raised about both international and national morality. The champions of national morality may wish to have a glance at the figures of Table IV taken from the data published by the World Bank.[13]

The message is straightforward; irrespective of their economic standard, governments spend much more money on defense than on health. Governments always provide justification for this, which is the behavior of other governments, but by the end of the day this philosophy might be suicidal.

Where do we go from here? What about our common future? Walt Whitman stated in 1856 that "the future is no more uncertain than the present" but, of course, he was not a demographer. The United Nations points out that yearly increments of the world population over the next decade will approach 97 million; 94% of them will be born in the developing world. Hence by the end of the century global population will increase by another billion, equivalent to the total world population at the beginning of the 19th century. But then? Will the growth ever come to an end? Yes, it will; all demographers agree that the world population will stabilize at the end of the next century. However, whether the plateau will be at 8, 10, or 14 (or more) billion people will largely depend on the number of years it will take to reach the replacement level of fertility worldwide, which is approximately 2.1 children per couple. If the replacement level of fertility is reached in 2010, global population will stabilize at about 8 billion by 2060. This is the United Nations' low projection from 1982 and very few people believe it to be realistic. If replacement level is reached in 2035, population will stabilize at 10 billion by 2095; this is the median projection that is used by most specialized agencies of the United Nations in their projections. However, if the rate is reached only in 2065, global population in 2100 will exceed 14 billion.[14]

Two remarks may be appropriate in this connection. The first is that these figures are demographic projections, and demographic projections are neither forecasts nor destiny. What they show is the most likely course of events, provided certain basic ("standard" or

Table II. Indicators of reproductive health

Category	*Millions (worldwide)*
No access to fertility regulation	400
Infertile couples	60-80
Induced abortions (yearly)	30-50
Maternal mortality (yearly)	0.5
Maternal morbidity (yearly)	25
Infants with low birth weight	20
Infant mortality (yearly)	4
Child mortality (1-5 yearly)	9
Sexually transmitted diseases (new cases yearly)	250

Source: United Nations and World Health Organization estimates, 1988 and 1990.[4]

Table III. Annual total of sexually transmitted diseases (new cases in 1990)

Disease	*New cases (millions)*
Trichomoniasis	120
Genital chlamydia	50
Genital papillomavirus	30
Gonorrhea	25
Genital herpes	20
Syphilis	3.5
Chancroid	2.0
Human immunodeficiency virus	1.0

Source: World Health Organization 1990.[11]

Table IV. Percentages of total central government expenditure allocated for health compared with education and defense (1986)

Type of economy	*Health*	*Education*	*Defense*
Low-income (excluding China and India)	3.6	9.8	17.7
Lower-middle	4.0	14.5	15.8
Upper-middle	5.1	10.2	10.3
Industrial market economies	12.9	4.5	16.4

Source: World Development Report, 1988.[13]

"critical") assumptions are fulfilled. The projections of the United Nations and also those of the World Bank are based on two such assumptions, namely, that mortality will continue to fall everywhere until life expectancy for women is 82 years and that fertility will eventually reach and stay at replacement levels everywhere. The other point is that in determinig the ultimate size of world population, fertility matters much more than mortality. Hence, if the decline in fertility is slower than expected, the projections of the ultimate size of world population may require a revision. Indeed, the United Nations Population Fund recently warned that if the current trends do not decrease, the medium estimate of 10.2 billion is likely to increase to 11.3 billion.

That the future is more uncertain than the present is evidenced by the most recent revision of the United Nations Population Division's world population projections for the year 2100. The latest medium projection is 11.2 billion, medium-high 17.6 billion, and medium-low 6.4 billion.[15]

In view of these and other considerations, I am deeply convinced that contraceptive prevalence is the key, not only to demographic development but also to improved reproductive and environmental health and to brighter economic and social development. That this is really the case is convincingly indicated by the studies conducted by Bongaarts et al.[16] of the Population Council in 96 countries. Those studies revealed a highly significant correlation between the total fertility rate (or, as a close approximation, the number of births per woman) and contraceptive prevalence. To every 10% increase in contraceptive prevalence, there is a corresponding decrease in fertility rate by 0.7.[1, 16]

The United Nations medium variant projection (with an ultimate population size of 10 billion or so) would therefore necessitate a decline of the current total fertility rate in developing countries (3.6 to 3.7) to a rate of 3.0 between the years 2000 and 2005 and to 2.4 between 2020 and 2025, which in turn will require an increase in contraceptive prevalence to 58% and 71%, respectively. In absolute numbers this would mean an increase in contraceptive users worldwide from the present 380 million couples to approximately 500 million by the year 2000, 650 million by the year 2010, and almost 900 million by 2025.[1, 4]

This is, of course, a big order and the first question is, is it feasible at all? Yes, it is, provided there is the political commitment and basic willingness of the population. Meanwhile, it took 58 years in the United States for fertility to decline from a rate of 6.0 to 3.5 (between 1842 and 1900); it took only 8 years in Thailand (between 1969 and 1977), thanks to the availability of modern technology, political will, and a genuine desire of the population to reduce family size.

What will it take to reach the big objective, the medium projection? First of all, money. The present yearly expenditure worldwide is around $4 billion, the major part of which is covered by a number of developing country governments, i.e., China, India, and Indonesia. It is estimated that the provision of family planning for half a billion couples by the year 2000 will incur a yearly cost between 8.4 and 11.4 billion dollars[3] and the $64,000 question is, who will provide the money? WHO reminds us that 80% of the world's gross domestic product is provided by a dozen countries, whereas 101 developing countries have readjustment programs and their per capita income today is much less than 10 to 15 years ago. However, the Development Assistance Committee of the 18 "rich" countries of Organization for Economic Cooperation and Development disburses some 48 billion dollars each year for official development assistance; that's where the money is.[17] Unfortunately, of the total donor development aid from industrialized nations to developing countries, only 1.3% goes for family planning. Why? Because there are no requests submitted by developing country governments. Why not? Because their political leaders receive one type of message from us and an entirely different type of message from some of our political and religious leaders. As the famous medicine man of the Kaiapo Indians of the Amazonas, Beptopup, said in an interview, "I am too old to understand the white man." Uncle Beptopup, you are not the only one.

Lack of money and lack of political commitment are important barriers to a rapid increase in contraceptive prevalence, but there are also others. The involvement of community leaders is as important as that of the central government. The root of the success of the Indonesian family planning program is the wholehearted support by religious leaders and traditional birth at-

tendants at the community level. Other important barriers may be cultural, educational, geographic (too long a distance to the nearest health center), or financial (too expensive for the family), or reasons related to the delivery of services or the scarcity of the available methods.[5]

Someone said that there has never been a generation that knew so much about man and so little about human nature. Therefore policy and sociobehavioral research in contraception and reproductive health are crucial, because "the effectiveness of any technology is shaped as much by the user as by the technology itself."[18]

Education is of paramount importance; in 1984 the World Fertility Survey demonstrated in the developing countries a significant correlation between the current use of efficient methods of contraception and the number of years of education.[19] The importance of these studies is underlined by a recent report from the United Nations Educational, Scientific, and Cultural Organization (Paris, 1988), indicating an illiteracy rate among women aged ≥15 of 48.9% in all developing countries and 78% in the 42 least-developed countries. The rate is 47.4% among Asian women and 64.5% among African women.[20] It is easy to see that there is a correlation between education and status of women. The WHO points out that one third of the households of the world are headed by women, and women constitute one third of the world's labor force. However, women are responsible for nearly two thirds of the total hours worked, receive only 10% of the world's income, and own <1% of its property.[9]

Another problem is the image of a "population mafia." A visitor to a rural health center may sometimes notice that a large variety of contraceptive drugs and devices are provided, but the simplest lifesaving materials and drugs are not available. The local population perceives this as a sign of conspiracy, whereas the simple truth is that contraceptives are provided by the development assistance agencies and essential drugs and materials should be provided by the national or regional government, but they are not.

Ready and easy access is also an important factor. Estimates for 1982 suggest that only in about 40% of developing countries does as much as half the national population have ready and easy access to some form of modern contraception.[1] In this context ready and easy access is defined as it should not take more than 2 hours per month and 1% of the monthly income of the family to obtain a month's supply of contraceptives.

The quality of family planning services also has a great significance. The best services are those that are integrated with maternal and child health care. The training and commitment of personnel are crucial for adequate counseling. Furthermore, there must be an adequate referral system and also a good follow-up system. Many developing countries are a long way from establishing an adequate service delivery.

A few words must be said about contraceptive development. What happens in the field? The need is increasing and the number and activities of the "actors" are decreasing. That contraceptive choice is too limited today is suggested by the outbreak of unwanted pregnancies among adolescents in many countries, by the high prevalence of induced abortions, and by the increasing reliance on sterilization, carried out at younger and younger ages. A greater variety of contraceptive methods is needed. What happens, however, is a significant reduction in contraceptive research and development. Carl Djerassi[21] points out that in 1970 there were 13 major pharmaceutical companies that conducted contraceptive research and development. By 1987 the number had dropped to four. Djerassi also stated that among the reasons for the retrenchment indicated by industrial leaders are factors like the especially expensive toxicologic requirements, the poor public image of the field caused by negative publicity, the fear of litigation, and the unavailability of insurance. In view of this development, the National Research Council in Washington warned in 1990 that "unless steps are taken to change public policy related to contraceptive development, contraceptive choice in the next century will not be appreciably different from what it is today."[22]

In an attempt to try to compensate at least in part for the diminishing industrial activities, in the 1970s a number of international and national contraceptive development programs were initiated, such as the Special Program of Research on Human Reproduction of the WHO, the Contraceptive Development Branch of the National Institute of Child Health and Human Development, the Population Council's International Committee on Contraceptive Research, Family Health International, The Contraceptive Research and Development Program (CONRAD), and the Program of the Indian Council of Medical Research (ICMR). All these programs are very cost-effective and show impressive achievements. However, in 1980 to 1983 their pooled yearly expenditure on contraceptive development was only around $25 million[23] whereas the industry's estimates for the cost of developing a new chemical entity into a drug are between $270 and $360 million.[24]

This brings us back to our starting point, the three different levels of reality—scientific, economic, and political. The World Commission on Environment and Development quotes Professor P. Lindbloom of the International Academy of Engineering, saying "the problems of today do not come with a tag marked energy, economy, or carbon dioxide or demography, nor with a label indicating a country or region. The prob-

lems are multidisciplinary and transnational or global. The problems are not primarily scientific and technical. In science we have the knowledge and in technology, the tools. The problems are basically political, economical and cultural."[25]

Almost 200 years ago, the second President of the United States, John Adams, wrote that, "Meanwhile all other sciences progressed, the art of governing did not develop. Its exercise today is hardly better than it was 3,000 or 4,000 years ago." This may not be valid today, at least not for all governments, but we are facing another, major political problem because global problems would require global solutions, which in turn would necessitate efficient global mechanisms. It is clear, however, as Jean Monnet said in his autobiography, that "the sovereign nations of the past can no longer solve the problems of the present. They cannot ensure their own progress or control their own future." Why not? Because "the traditional forms of nation sovereignty are increasingly challenged by the realities of ecological and economical interdependence.[25] And, whatever happens in organizational terms with our global reality, it seems to me obvious that sustaining a global population of 14 billion human beings or more will require a very high level of technical civilization and, an intimately interconnected world economy. As Adlai Stevenson hoped, there should be a general recognition that "our needs for our fellow men and women far outweigh our arguments with them."

In the 1970s there was a symposium entitled "Can We Survive Our Future?"[26] The answer is that of course we can. There is a real future for man and, as Jawaharlal Nehru said, the future belongs to science.[27] The future is created in the minds of the new generation of scientists and that future is truly international. However, to see the real impact of science, one needs a broad historic perspective. In 1944 Winston Churchill said that "the longer you can look back, the further you can look forward."[28] R.L. Lesher and G.J. Howick, in a National Aeronautics and Space Administration report in 1966, look back 50,000 years. They stated that "800 life spans can bridge more than 50,000 years; but of these 800 people, 650 would have spent their lives in caves or worse. Only the last 70 had any truly effective means of communicating with one another, and only the last six ever saw a printed word. Only the last four could measure time with any precision and only the last two used an electric motor; the vast majority of the items that make up our material world were developed within the life span of the eight hundredth person."[29]

All of us could add a highly significant milestone to that list, be it telephone, television, computer revolution, landing on the moon—there is no end to it. One may also reflect on the fact that at least 90% of the drugs featured in our daily prescriptions were developed by the eight hundredth person. Indeed, in 1977, Henderson remarked that "somewhere between 1910 and 1912 in this country . . . a random patient with a random disease consulting a doctor chosen at random had for the first time in history of mankind, a better than 50-50 chance of profiting from the encounter." So why do we despair? There is a future for man and that future is called science.

However, we must go a step further. There is a classical Latin quotation, *tempora mutantur, nos et mutamur in illis,* meaning that times change and we change with them, attributed to Emperor Lothar I of the ninth century. The problem with this quotation is that it is true with one qualification—we don't change with the same speed. The changes around us are accelerating so rapidly that our perception of these changes and of their consequences is lagging behind. In the final analysis, all of us are prisoners of our perceptions, watching shadows on the walls of Plato's cave; therefore people's perceptions of reality may have a controlling influence on the future of the human race. To a scientist, many of those perceptions are utterly naive or even dangerous. At a meeting of Nobel laureates convened by President Mitterrand to celebrate the bicentennial of the French revolution, Elie Wiesel said the following: "Scientists have provided mankind with the means of destruction. It is up to them now to find the means for saving mankind." Great! And what will the other 5.3 billion human beings do? Wait and see?

I see a monumental challenge, to be faced by the international scientific community and by every single physician, for that matter: to try to change the perception of priorities and their consequences by the people and their governments. Can it be done? Yes, it can, but it will require more effort than to discover the brave new world in the outer spaces of the universe. Why? Because, as Werner Heisenberg said in 1971, "Reason is a very limited faculty of the human mind." If so, can it be done at all? Yes, it can, because reason is a limited faculty, not an absent faculty of the mind. Therefore a very great global effort is needed.

What should be our message? I would like to suggest one—telling people of the world that fundamentalism and obscurantism will not improve the quality of life for future generations; only science and technology have proved the ability to do so. Why is it so? Because, as Peter Drucker[31] said "We don't know the answers but we know the issues." And I would like to add that we also know which issues are researchable and how.

My last remark, if I may say this, is: In the final analysis hope is the quintessence of the human condition. Therefore it is crucial to generate fresh hope

among young scientists and to stimulate and support their efforts in the acquisition, systematization, and dissemination of positive knowledge, which is the key to a brighter future.

I started with a quotation from William Faulkner. Let me close with another of his sayings:

> I decline to accept the end of man . . .
> I believe that man will not merely endure;
> He will prevail . . . .

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Evidence of embryo- and trophoblast-toxic cellular immune response(s) in women with recurrent spontaneous abortion

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OBJECTIVE: The purpose of this study was to determine whether reproductive antigens stimulate lymphocytes and macrophages from women with recurrent abortion to secrete factors that are toxic to preimplantation embryos or trophoblast cells in vitro.

STUDY DESIGN: Mononuclear cells were isolated from 30 fertile controls and 300 nonpregnant women being evaluated for recurrent abortion. Supernatants generated from these cells after separate culture with sperm and trophoblast antigen extracts were added to two-cell mouse embryo cultures and trophoblast proliferation assays. Toxicity was assumed when the median percentage of embryos developing to blastocysts or trophoblast proliferation was ≤50% of control values. Both parametric and nonparametric statistical methods were used where appropriate.

RESULTS: Mouse embryo development and/or trophoblast proliferation were significantly inhibited by supernatants from trophoblast and/or sperm antigen-activated peripheral blood leukocyte cultures from a majority of 300 women with recurrent abortion but not from 30 women with normal reproductive histories. The mouse blastocyst development assay was more sensitive than the trophoblast proliferation assay in determining toxic factor production. Embryo-toxic factors were produced by activated leukocyte cultures from 90% of 180 women with a history of recurrent abortion of unexplained etiology, whereas trophoblast-inhibitory factors were detected in 50% of women from the same group. The embryo-toxic factor(s) was heat labile, had a molecular weight(s) between 10 and 30 kd, and was absorbed out by passage through affinity columns containing anti–interferon gamma beads.

CONCLUSION: We conclude that recurrent abortion in some women is associated with embryo- and/or trophoblast-toxic factor production in response to stimulation by sperm or trophoblast antigens and that the principal factor may involve the 18 kd, heat-labile, T-lymphocyte cytokine interferon gamma. This study suggests a new cause of recurrent abortion. (AM J OBSTET GYNECOL 1992;166:1044-52.)

Key words: Cellular immune response, embryo, trophoblast, recurrent abortion

Recurrent spontaneous abortion, defined as three or more pregnancy losses before 20 weeks' gestation, occurs in approximately one in 300 pregnancies.[1] Structural chromosomal rearrangements in the couple experiencing recurrent abortion are a well-established cause.[2] Other potential etiologies of recurrent abortion are controversial, although many associations have been made, including anatomic or müllerian anomalies, endocrine disorders, and infections.[3] The cause is unexplained in 40% to 60% of couples.[4,5] Immunologic factors have been implicated in many of these cases.[6]

The cellular immune response to reproductive antigens (i.e., sperm and trophoblast) has been relatively unexplored even though human decidua contains abundant immune cells capable of responding to antigenic stimulation,[7] and many soluble products of activated lymphocytes and macrophages adversely affect embryo development[8] and trophoblast proliferation[9] in vitro. The purpose of this study was to determine whether sperm or trophoblast antigens can stimulate immune cells from women with recurrent abortion to secrete factor(s) that are detrimental to embryo development and trophoblast proliferation in vitro as a possible mechanism for their reproductive failure.

Methods

Study patients. The subjects in this study were 300 reproductive-aged, nonpregnant women undergoing evaluation for recurrent abortion at the Brigham and Women's Hospital Reproductive Endocrinology and Infertility Clinic between July 1, 1986, and June 31, 1989. All subjects were consecutively enrolled and no one refused participation. The women were between 22 and 42 years old and had a history of at least three prior spontaneous abortions with or without a stillbirth,

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ectopic pregnancy, or live birth. These women were grouped by clinical history as follows: women with primary abortion (no live births), women with secondary abortion (abortions subsequent to live births or stillbirths), women with previous ectopic pregnancy, or women with other criteria (abortions interspersed with live births). After a complete medical, surgical, and social history was obtained from the couple and a thorough physical examination was performed on the woman, all couples had peripheral blood chromosome assessment by standard banding techniques. All women underwent hysterosalpingography followed by hysteroscopy-laparoscopy when indicated. Ovulation was assessed by a late-luteal-phase endometrial biopsy with concomitant serum progesterone determination. When the endometrial biopsy specimen was out of phase according to the criteria of Noyes et al.,[10] the suspected ovulation disorder was confirmed by repeating the biopsy in a subsequent cycle. In addition, all women had cervical cultures for mycoplasma and ureaplasma, and blood was obtained for thyroid-stimulating hormone, thyroxine, prolactin, anticardiolipin antibody, and lupus anticoagulant (Russell viper venom time) determinations. Cervical mucus and serum were also obtained for antisperm antibody assessment. After the preceding evaluation, study patients were categorized as having either a genetic, anatomic, endocrine, infectious, or humoral immune cause for their reproductive failure. Peripheral blood was obtained from all women on their initial visit for embryo- and trophoblast-toxic factor(s) testing.

Peripheral blood was also obtained from a comparison group of 30 nonpregnant, paid volunteer women who were randomly selected from the Obstetrical Nursing Service. Women in this control group were of reproductive age (23 to 41) with a history of at least three prior uncomplicated term deliveries and no history of either spontaneous abortion, ectopic pregnancy, or stillbirth. None of the women in the study were taking medication at the time of blood collection.

Embryo- and trophoblast-toxic factor assay

Reproductive antigen preparation. Trophoblast antigen extracts were prepared from the human gestational choriocarcinoma cell lines BeWo and Jeg-3 (American Tissue Type Collection, Bethesda, Md.). These cells were grown in culture flasks by standard culture techniques until confluence. After trypsinization, the cells were washed twice in Hanks' balanced salt solution without calcium or magnesium (Gibco, Grand Island, N.Y.), and cells excluding trypan blue were counted on a hemocytometer (viability always >90%). The cells were diluted to 10^7 cells per milliliter in tissue culture media comprising Roswell Park Memorial Institute 1640 medium (RPMI; Gibco), 10% fetal calf serum, 0.2 mmol/L glutamine, 100 IU/ml benzylpenicillin potassium, and 100 μg/ml streptomycin sulfate (Sigma, St. Louis). Cells were disrupted in a dounce homogenizer, and after centrifugation at 400*g* for 10 minutes, the membrane-enriched trophoblast antigen supernatant was divided into aliquots and stored at −70° C until use.

Semen was obtained from fertile donors by masturbation into sterile containers, and motile sperm were isolated by percoll density gradient centrifugation.[11] Aliquots of 10^7 sperm per milliliter in tissue culture media were dounce homogenized. The suspension was then centrifuged at 400*g* for 10 minutes and the membrane-enriched sperm antigen supernatant was divided into aliquots and stored at −70° C until use.

Supernatant preparation. Mononuclear leukocytes were separated from blood by Ficoll-Hypaque (Pharmacia, Uppsala) centrifugation. Isolated cells were washed twice and resuspended in RPMI medium. Viable mononuclear cells excluding trypan blue were counted on a hemocytometer (viability always >90%) and diluted in tissue culture medium to a concentration of 10^6 cells per milliliter. Leukocyte suspensions were then divided into three 50 ml tissue culture flasks (Becton Dickinson, Lincoln Park, N.J.) containing 10^7 leukocytes per 10 ml of medium in each loosely capped flask. One milliliter of either trophoblast antigen suspension, sperm antigen suspension, or tissue culture medium alone was added to each flask. After a 72-hour incubation at 37° C in 5% carbon dioxide and 95% air, the cell cultures were transferred to separate 15 × 95 mm polystyrene round-bottom tubes with caps (Becton Dickinson) and pelleted by centrifugation at 400*g* for 10 minutes. The cells were next washed once in RPMI medium and once in Whitten's medium[12] and then resuspended in 1 ml of Whitten's medium supplemented with antibiotic-antimycotic solution (Gibco) that had been previously passed through a 0.45 μm filter (Corning Glass, Corning, N.Y.). The pH of the medium was adjusted before use to 7.3 with carbon dioxide gas and supplemented with 0.3% bovine serum albumin (Miles Scientific, Naperville, Ill.). After an additional 12-hour incubation at 37° C in 5% carbon dioxide and 95% air, the three suspensions from each original sample were centrifuged at 100*g* for 10 minutes. The three supernatants were each filtered through a 0.22 μm Millex-GS filter unit (Millipore, Bedford, Mass.) and stored at −70° C until use.

Embryo development assay. Embryo culture was performed as previously described.[8] Briefly, two-cell embryos were harvested from CD-1 female mice (Charles River, Kingston, Ontario) 44 hours after human chorionic gonadotropin administration. Embryos were cultured in 20 μl drops containing a 1:1 dilution of study supernatant in Whitten's medium–0.3% bovine serum albumin under carbon dioxide–equilibrated paraffin oil in Falcon tissue culture dishes (Becton Dickinson). Media and supernatants were equilibrated overnight in

Table I. Mean age of women with and without recurrent abortion and median percent of embryo development and trophoblast proliferation in response to trophoblast or sperm antigen–activated leukocyte supernatants by category and cause of spontaneous abortion

	Age at abortion (yr)					*Embryo development and trophoblast proliferation*	
						ED-T	
	No.	*%*	*Mean*	*SD*	*SEM*	*Median (%)*	*10th and 90th percentiles*
No history of abortion	30		37.5	3.1	0.6	63	54,82
History of abortion	300		35.9	3.4	0.2	45	10,72
Category of abortion							
Primary	215	72	36.1	3.2	0.2	45	15,72
Secondary	50	17	35.1	4.0	0.6	45	5,71
Ectopic pregnancy	7	2	37.7	3.4	1.3	45	18,72
Other	28	9	35.4	4.2	0.8	43	0,63
Cause of abortion							
Unknown	180	60	36.4	3.4	0.3	36	0,71
Endocrine	51	17	36.0	3.0	0.4	45	18,70
Anatomic	29	10	34.7	3.3	0.6	60	50,72
Infection	14	4	32.9	4.1	1.1	54	27,81
Humoral immune	11	4	36.8	1.5	0.5	36	20,72
Genetic	15	5	34.5	3.6	0.9	60	18,81

ED-T, Embryo development in response to trophoblast antigen–activated leukocyte supernatants; *ED-S,* embryo development in response to sperm antigen–activated leukocyte supernatants; *TP-T,* trophoblast proliferation in response to trophoblast antigen–activated leukocyte supernatants; *TP-S,* trophoblast proliferation in response to sperm antigen–activated leukocyte supernatants.

5% carbon dioxide and 95% air before the addition of embryos. Embryos cultured in Whitten's medium–0.3% bovine serum albumin alone served as an additional control. At least 11 embryos were cultured in each 20 μl drop. Embryos were assessed for normal development after 4 days in culture (100 hours after human chorionic gonadotropin administration) by the criteria described by Ducibella.[15] The experimental end point was the percentage of normal blastocysts in each culture after 4 days of development in vitro. The sensitivity and specificity of the assay were maximal when the median percentage of embryos advancing to the blastocyst stage of development was ≤50% of control values. The intraassay and interassay coefficients of variation were 8% and 12%, respectively, for the embryo-toxic bioassay.

Trophoblast proliferation assay. Human gestational choriocarcinoma cells (10^4 cells per 0.1 ml tissue culture medium) were plated in triplicate into 96-well, flat-bottom microtiter trays (Becton Dickinson). Two hours after cell plating, 0.1 ml of leukocyte supernatant or media alone was added (four wells per test solution). After a 2-day incubation at 37° C in 5% carbon dioxide and 95% air, 0.5 μCi of tritiated thymidine (13.1 Ci/mmol; New England Nuclear, Boston) was added to each well. Cells were harvested at 72 hours after trypsinization onto glass fiber filter paper with a MASH II automatic sample harvester (Microbiological Assoc., Los Angeles). Viability of control cultures was assessed before harvesting by means of trypan blue exclusion (viability always >90%). Filter paper disks corresponding to each well were air dried, transferred to scintillation counting vials, and suspended in 2 ml of Betafluor scintillation cocktail (National Diagnostics, Summerville, N.J.). Radioactivity (counts per minute) was measured in a liquid scintillation counter (Beckman Instruments Inc., Fullerton, Calif.) (efficiency of the system 45%), and the mean ± SD of each set of triplicate cultures was calculated. The data are presented as percentages of culture medium control. Trophoblast toxicity was assumed when the median percentage of trophoblast proliferation was ≤50% of control values.

Toxic factor characterization. Trophoblast antigen–stimulated leukocyte supernatants from 15 patients that adversely affected embryo development were subjected to three different methods, to characterize the factor(s) responsible for embryo toxicity: molecular weight dialysis, heat inactivation, and affinity column purification. The supernatants were dialyzed for 12 hours against three changes of Whitten's medium–0.3% bovine serum albumin in Spectrapor membrane tubing (Spectrum Medical Industries, Los Angeles) with molecular weight limits of 3500, 10,000, or 30,000 and then tested in the mouse embryo development assay. In separate experiments aliquots of these supernatants were heat treated at 56° C for 1 hour or passed through an affinity column containing anti–interferon gamma–coated beads (Endogen, Boston) before testing in the mouse embryo development assay. Five leukocyte supernatants not exhibiting an adverse effect and me-

Embryo development and trophoblast proliferation

ED-S		*TP-T*		*TP-S*	
Median (%)	*10th and 90th percentiles*	*Median (%)*	*10th and 90th percentiles*	*Median (%)*	*10th and 90th percentiles*
72	54,81	70	60,90	75	60,90
54	9,72	60	20,80	70	45,80
54	9,72	60	20,80	70	45,80
48	9,77	60	23,80	65	45,80
36	27,72	70	40,80	60	50,80
52	27,80	65	10,80	70	60,80
43	9,72	60	20,80	70	43,80
54	27,81	60	10,80	80	60,80
60	40,81	70	60,90	70	60,90
54	9,90	60	40,70	60	40,70
63	20,72	80	50,80	80	30,80
54	18,72	60	20,70	60	40,70

dium alone were used as controls in each of these experiments.

Statistical analysis. We compared the mean age distribution between the 300 women with recurrent abortion and the 30 control subjects with a two-sample *t* test for independent samples and assumed equal or unequal variances based on the F distribution.[14] Differences in mean ages by different categories of abortion and spontaneous abortion causes were assessed through analysis of variance. The percentages of mouse blastocyst development or trophoblast proliferation in supernatants failed the test of normality with the Shapiro-Wilk W statistic[15]; therefore median percentages were compared between the 300 women with recurrent abortion and the 30 controls overall and within each of the categories of abortion and spontaneous abortion causes with the use of nonparametric tests of medians and the Wilcoxon rank sum test.[16] All directional *p* values were two tailed.

Results

Women with recurrent abortion were 80% white, 10% Hispanic, 8% black, 1% Native American, and 1% Asian. The 30 fertile controls consisted of 25 white, 3 Hispanic, and 2 black women. The 300 women evaluated for recurrent abortion had experienced 1367 prior pregnancies, 1213 of which resulted in spontaneous abortion (median 4, range 3 to 25). Fetal loss occurred during the first trimester in 88%, whereas 12% of the abortions occurred during the second trimester (before 17 weeks of gestation).

The control group of women without a history of abortion were slightly older than women with a history of spontaneous abortions (37.5 ± 3.1 vs 35.9 ± 3.4, Table I). Among the 300 women with recurrent abortion, 215 women (72%) had primary abortions, 50 women (17%) had secondary abortions, and the remaining 35 women (11%) could not be identified as having either primary or secondary abortions. Seven of these women had a total of 9 ectopic pregnancies (all tubal gestations), and the other 28 women experienced recurrent abortions interspersed with live births.

The cause of recurrent abortion was unexplained in 180 (60%) women. The remaining 120 women (40%) had recurrent abortion presumably as a result of endocrine abnormalities (17%), anatomic anomalies (10%), infections (5%), humoral immune disturbances (4%), or chromosomal aberrations (5%). There was no correlation between the average age of the women and etiologic categories, except in women with an infectious cause (positive cervical culture for mycoplasma or ureaplasma), who were significantly younger than the other women with a history of spontaneous abortion (32.9 ± 4.1 vs 35.7 ± 1.0, $p < 0.01$).

There was no difference between effects of supernatants derived from unstimulated peripheral blood mononuclear cells from women in any category and control medium in either the embryo development or trophoblast proliferation assays (data not shown). Embryo development and trophoblast proliferation in response to trophoblast antigen–activated leukocyte supernatants and sperm antigen–activated leukocyte supernatants, are shown in Table I. Neither trophoblast antigen–activated nor sperm antigen–activated leu-

Table II. Sensitivity and specificity for recurrent abortion by assay and antigenic factor

	Women with abortions (No.)	*Women without abortions (No.)*	*Sensitivity (%)*	*Specificity (%)*
ED-T				
Pos. (≤50%)	188	0	63	100
Neg. (>50%)	112	30		
ED-S				
Pos. (≤50%)	153	1	51	97
Neg. (>50%)	147	29		
TP-T				
Pos. (≤50%)	95	0	32	100
Neg. (>50%)	205	30		
TP-S				
Pos. (≤50%)	40	0	13	100
Neg. (>50%)	260	30		

ED-T, Embryo development in response to trophoblast antigen–activated leukocyte supernatants; *ED-S*, embryo development in response to sperm antigen–activated leukocyte supernatants; *TP-T*, trophoblast proliferation in response to trophoblast antigen–activated leukocyte supernatants; *TP-S*, trophoblast proliferation in response to sperm antigen–activated leukoctye supernatants.

kocyte supernatants from the 30 fertile women adversely affected embryo development or trophoblast proliferation. Trophoblast and sperm antigen extracts alone had no effect on either embryo development or trophoblast proliferation. Neither race nor ethnicity was associated with either the production of toxic factors or the various causes of recurrent abortion. Both embryo development and trophoblast proliferation in the presence of trophoblast antigen–activated and sperm antigen–activated leukocyte supernatants were decreased in women with recurrent abortion as compared with fertile controls. In the 300 patients with recurrent abortion, embryo development was more often and more severely affected than trophoblast proliferation in the presence of both sperm antigen–activated and trophoblast antigen–activated leukocyte supernatants (effects of trophoblast and sperm antigen–activated leukocyte supernatants on embryo development versus trophoblast proliferation $p < 0.01$). Among the four categories of women with abortions in Table I, embryo development was more adversely affected by trophoblast antigen–activated leukocyte supernatants than by sperm antigen–activated leukocyte supernatants, except in those women with a prior ectopic pregnancy.

Embryo development in the presence of trophoblast antigen–activated or sperm antigen–activated leukocyte supernatants from women with an unknown cause of abortion was significantly less than embryo development in comparable supernatants from women with other causes ($p < 0.01$). Embryo development was also observed to be lower in trophoblast antigen–activated and sperm antigen–activated leukocyte supernatants from women with recurrent abortion caused by an endocrine or humoral immune abnormality ($p < 0.001$) but not in those from the two groups of women with an anatomic or genetic etiology of recurrent abortions. In the two women with antisperm antibodies, embryo development was unaffected by sperm antigen–activated leukocyte supernatants but was adversely affected by trophoblast antigen–activated leukocyte supernatants. However, within the anatomic group embryotoxic factors were produced in women with a diagnosis of endometriosis as their only abnormal finding in response to stimulation by both trophoblast and sperm antigens ($p < 0.05$). In the same group trophoblast proliferation was also significantly inhibited in response to trophoblast antigen–activated leukocyte supernatants from women with endometriosis ($p < 0.05$). A woman with a pericentric inversion of chromosome 9 had low embryo development in response to both trophoblast antigen–activated and sperm antigen–activated leukocyte supernatants and low trophoblast proliferation in response to trophoblast antigen–activated leukocyte supernatants. Otherwise, cytogenetic abnormalities were not associated with either embryo or trophoblast toxicity.

The sensitivity and specificity of the bioassays were greatest when embryo development and trophoblast proliferation were ≤50% of control values (Table II). Only one woman in the fertile control population had a value <50%. This woman had 45% blastocyst development in the presence of supernatant from her sperm antigen–activated leukocyte culture. Using 50% of control values as our cutoff, we detected women with recurrent abortion as follows: for embryo development assays, 63% using trophoblast antigen–activated and 51% using sperm antigen–activated leukocyte super-

Table III. Outcome of embryo development and trophoblast proliferation assays by cause of abortion

	≤50% ED-T or ED-S (pos.)		*>50% ED-T or ED-S (neg.)*		
	≤50% TP-T or TP-S	*>50% TP-T or TP-S*	*≤50% TP-T or TP-S*	*>50% TP-T or TP-S*	*Total No. of patients*
All women with recurrent abortion	109	127	4	60	300
Suspected cause					
Unknown	72	88	2	17	180
Endocrine	18	21	1	11	51
Anatomic	2	8	0	19	29
Infection	7	3	0	4	14
Humoral immune	3	5	0	3	11
Genetic	6	2	1	6	15
Type of recurrent abortion					
Primary	80	88	3	44	215
Secondary	18	23	1	8	50
Ectopic pregnancy	2	4	0	1	7
Other	9	12	0	7	28

ED-T, Embryo development in response to trophoblast antigen–activated leukocyte supernatants; *ED-S*, embryo development in response to sperm antigen–activated leukocyte supernatants; *TP-T*, trophoblast proliferation in response to trophoblast antigen–activated leukocyte supernatants; *TP-S*, trophoblast proliferation in response to sperm antigen–activated leukocyte supernatants.

natants; for trophoblast proliferation, 32% using trophoblast antigen–activated and only 13% using sperm antigen–activated leukocyte supernatants. Only one (3%) of the fertile controls would have been falsely classified as having recurrent abortion with sperm antigen–activated leukocyte supernatant in the embryo development assay, and none would have been falsely classified with the other assay-supernatant combinations.

The embryo development assay was more sensitive than the trophoblast proliferation assay in predicting the propensity for abortion. Only four of 300 women had >50% embryo development when trophoblast proliferation was ≤50% (Table III). If only trophoblast proliferation had been used to assess toxic factor production, 127 women would have been falsely screened as negative. Only 113 women produced toxic factors in response to reproductive antigens in the trophoblast proliferation assay compared with 236 women who tested positive with the embryo development bioassay (Table III).

In Table IV we considered a positive test for recurrent spontaneous abortion to be ≤50% of embryo development in response to either trophoblast or sperm antigen–activated leukocyte supernatants. This allowed us to detect 79% of women with recurrent abortion while still maintaining an extremely low number of false-positive results (3%). This propensity to generate embryo-toxic factors in response to either trophoblast or sperm antigen stimulation was more pronounced in women with an unknown cause as compared with a known cause for their abortion. The presence of embryo- and trophoblast-toxic factors was not influenced by a change in partners because six women in whom toxic factors were produced in response to sperm-antigen stimulation (n = 4) and trophoblast antigen stimulation (n = 2) experienced recurrent abortions with both their prior and current husbands.

The effects of embryo-toxic supernatants on embryo development after molecular weight dialysis indicated that the factor(s) responsible for embryo toxicity in all 15 supernatants tested was between 10 and 30 kd. The embryo-toxic effect of the supernatants was abrogated by heat treatment to 56° C for 1 hour. In 12 of 15 embryo-toxic supernatants the factor(s) was absorbed on affinity columns containing anti–interferon gamma beads. Passage of control media through the anti–interferon gamma column had no effect on embryo development.

Comment

Viable pregnancy is the result of a highly selective process because 10% to 20% of all conceptions end in detectable spontaneous abortion.[3] Recurrent spontaneous abortion is an important clinical problem affecting the reproductive health of up to 1% of women who are gravida ≥3.[17]

Women with recurrent abortion have been categorized as having either primary or secondary abortions depending on whether they have never achieved a viable pregnancy or their first pregnancy resulted in a live birth followed by recurrent abortion.[18] In this study 11% (n = 35) of the women experiencing recurrent abortion defied conventional classification. The use of the terms primary and secondary abortion is therefore limited because of their failure to include women who have had spontaneous abortions interspersed with either live births or ectopic gestations.

This report of 300 couples with recurrent abortion is the largest completed series to date. Earlier reports

Table IV. Sensitivity and specificity of the embryo development assay for predicting spontaneous abortion in women with known and unknown causes of their abortions

	Women with abortions (No.)	*Women without abortions (No.)*	*Sensitivity (%)*	*Specificity (%)*
All women				
ED-T or ED-S ≤50% (pos.)	236	1		
			79%	97%
ED-T and ED-S >50% (neg.)	64	29		
Cause unknown				
ED-T or ED-S ≤50% (pos.)	161	1		
			89%	97%
ED-T and ED-S >50% (neg.)	19	29		
Cause known				
ED-T or ED-S ≤50% (pos.)	75	1		
			63%	97%
ED-T and ED-S >50% (neg.)	45	29		

ED-T, Embryo development in response to trophoblast antigen–activated leukocyte supernatants; *ED-S*, embryo development in response to sperm antigen–activated leukocyte supernatants.

of 100,[4] 155,[19] and 195[5] couples with recurrent abortion described parental chromosomal errors in 12%, 15%, and 3% of their study patients, respectively. Our finding of a chromosomal abnormality in 5% of couples with recurrent abortion is in agreement with the 3% prevalence rate observed in the next largest reported series of 195 couples.[5] The discrepancy in the percentage of individuals with various causes between studies may be due to differences in sample size, differences in diagnostic criteria, and inherent bias afforded by physician referral patterns of patients with recurrent abortion.

Nongenetic causes of recurrent abortion are difficult to ascribe, although numerous associations have been made with anatomic, endocrine, infectious, and humoral immune abnormalities.[3] Anatomic anomalies occurred in 10% of the women in our study with recurrent abortion, which is similar to the 15% rate reported in two of the other series of 100 and 195 patients.[4,5] A diagnosis of septate uterus accounted for the majority of women in our study with an anatomic cause. Timing of fetal loss in these women was equally distributed between the first and second trimesters. Uterine anomalies have been primarily associated with second-trimester fetal loss.[20] However, first-trimester losses may occur as a result of vascular insufficiency and inadequate endometrial development resulting in abnormal placentation. Endocrinologic dysfunction occurred in 17% of our patients, which is also similar to findings in other published series.[4,5] The majority (88%) of our patients with an endocrinologic abnormality had luteal phase insufficiency. The significance of luteal phase deficiency in recurrent abortion, like other nongenetic associations, is controversial.[3] However, every attempt was made in this study to eliminate possible contributing factors to recurrent abortion. Thus a series of women was identified with an otherwise unexplained cause of reproductive failure to test the hypothesis that reproductive antigens stimulate lymphocytes and macrophages from some women with recurrent abortion to secrete soluble factors that are capable of inhibiting embryo development and trophoblast proliferation as a possible mechanism for spontaneous abortion.

Both sperm and trophoblast antigens in this study stimulated peripheral blood lymphocytes and macrophages from many of the women with recurrent abortion to secrete factors that adversely affected embryo development and trophoblast proliferation in vitro. The trophoblast antigen used in this study was derived from the choriocarcinoma cells lines BeWo and Jeg-3. These cell lines were chosen as the trophoblast antigen model for three reasons: (1) They are homogeneous trophoblast cell lines uncontaminated by other cellular constituents such as stromal mesenchymal cells or lymphoid and myeloid populations that are always associated with normal placental trophoblast[21]; (2) like normal trophoblast, they are devoid of classic major histocompatibility complex class I and II antigens[22]; (3) they are established antigenic models of early normal trophoblast because of their invasive capabilities and cell surface antigen profiles that are similar to those of early normal human trophoblast.[23,24] Placental trophoblast isolation techniques have to date been unable to achieve absolute purity, and even low levels (<2%) of allogeneic immune cell contamination are sufficient to cause a mixed lymphocyte reaction if these trophoblast preparations are cocultured with leukocytes from a different individual or with other cell lines expressing either major histocompatibility complex class I or II antigens (unpublished observations). This is important to avoid because supernatants generated from mixed lymphocyte cultures even from fertile individuals contain factors capable of inhibiting embryo development in vitro.[8] This is not surprising because abundant concen-

trations of cytokines including interferon gamma and tumor necrosis factor-α can be isolated from mixed lymphocyte culture supernatants.[25] There were no observed differences between BeWo and Jeg-3 antigen preparations in terms of their ability to stimulate embryo-toxic factor release from activated leukocytes of susceptible individuals. Therefore these two trophoblast antigen sources were used interchangeably.

Antibody responses directed against sperm antigens are well described as factors contributing to female infertility.[26] In addition to affecting prefertilization events, antisperm immunity may also mediate early embryonic loss.[27] Cellular immunity to sperm has also been suggested to participate in infertility mechanisms[28] and has been speculated to contribute to abortion.[29] Many studies have revealed the presence of sperm antigens on the fertilized egg, early embryo, and developing trophoblast.[30] Therefore it may not be surprising to find, within a group of women with recurrent abortion, those who produce either embryo-toxic or trophoblast-toxic factors in response to sperm antigen stimulation.

Women with unexplained cause of recurrent abortion had a high incidence of embryo-toxic and trophoblast-toxic factor production in response to reproductive antigen stimulation. Of 180 women with an unexplained cause for their recurrent abortions, approximately 90% had production of embryotoxic (<50% embryo development, $n = 161$) and/or trophoblast-toxic (<50% trophoblast proliferation, $n = 75$) factors in response to either trophoblast antigen or sperm antigen activation (Table III). Approximately 78% of the women with a presumed endocrinologic abnormality as the cause for their recurrent abortions also had production of these toxic factors. The majority of these women (88%) had a diagnosis of luteal phase deficiency. It is possible that the generation of embryotoxic factors is associated with luteal phase deficiency and contributes to or is responsible for abortion in these women. Luteal phase deficiency may even be a marker for embryo-toxic factor production because many women with luteal phase deficiency have increased concentrations of T lymphocytes in the endometrium that are capable of cytokine secretion, as compared with concentrations in endometrial biopsy specimens from women without luteal phase deficiency.[31] Similarly, many of the women in this study who were found to have a humoral immune (antibody) abnormality as a potential cause for recurrent abortion also had production of embryo-toxic factors in response to reproductive antigen stimulation. Women with antisperm antibodies in this study did not have embryo-toxic factors produced in response to sperm antigen stimulation but instead in response to trophoblast antigens (data not shown). This may be attributable to either lack of association between humoral and cellular immunity to sperm antigens or cross reactivity of sperm antigens with dominant antigens in the trophoblast extract preparation. However, the number of women with antisperm antibodies in this study was too small for definitive conclusions. Women with either an anatomic or a genetic cause of recurrent abortion were less likely to have toxic factors produced in response to reproductive antigen stimulation than women with other causes of abortions. The finding of embryo-toxic and trophoblast-toxic factors in some women with endometriosis is interesting. However, it may not be uncommon for individuals to have more than one cause contributing to reproductive failure. Cause-and-effect relationships are difficult to ascribe; it is possible that in some women embryo-toxic factor production was related to exposure to degenerating or necrotic fetal-placental tissue that occurred during a previous pregnancy failure. However the cytotoxic cellular immune mechanism is initiated, embryo-toxic factor production could be the cause of subsequent abortion.

Our study indicates that the preimplantation embryo is a vulnerable target of cytotoxic factors produced by activated leukocytes from many women with recurrent abortion, yet the clinical histories of these patients indicate that their pregnancies aborted after the implantation stage. We do not know at the present time to what extent immunologic embryo-toxic factors contribute to idiopathic infertility, but this possibility is currently under investigation. In patients with recurrent abortion detrimental effects of immune activation could be manifested later in gestation because of a delayed response and/or inadequate antigenic stimulus provided by the preimplantation embryo.

Female reproductive tissues contain abundant immune cells capable of responding to antigen stimulation.[7, 32] Products of activated immune cells, collectively termed cytokines, affect many reproductive processes in vitro. The T-lymphocyte cytokine interferon gamma inhibits both embryo development[8] and trophoblast proliferation[9] over a wide concentration in vitro. Interestingly, one form of interferon gamma has a molecular weight of 17,000 kd, which is comparable to that of the embryo-toxic factor isolated in our molecular weight dialysis experiments. Furthermore, interferon gamma is heat labile.[33] Our finding that embryo toxicity was abrogated in 12 of 15 supernatants after passage through an affinity column containing antibody against interferon gamma suggests that the factor responsible for embryo toxicity in these supernatants may be interferon gamma or a soluble immune cell factor that either has strong affinity for this cytokine or works in synergy with interferon gamma. A number of products of activated macrophages have also been reported to have toxic effects on preimplantation embryos and trophoblast cell lines.[8, 9] These or other, as

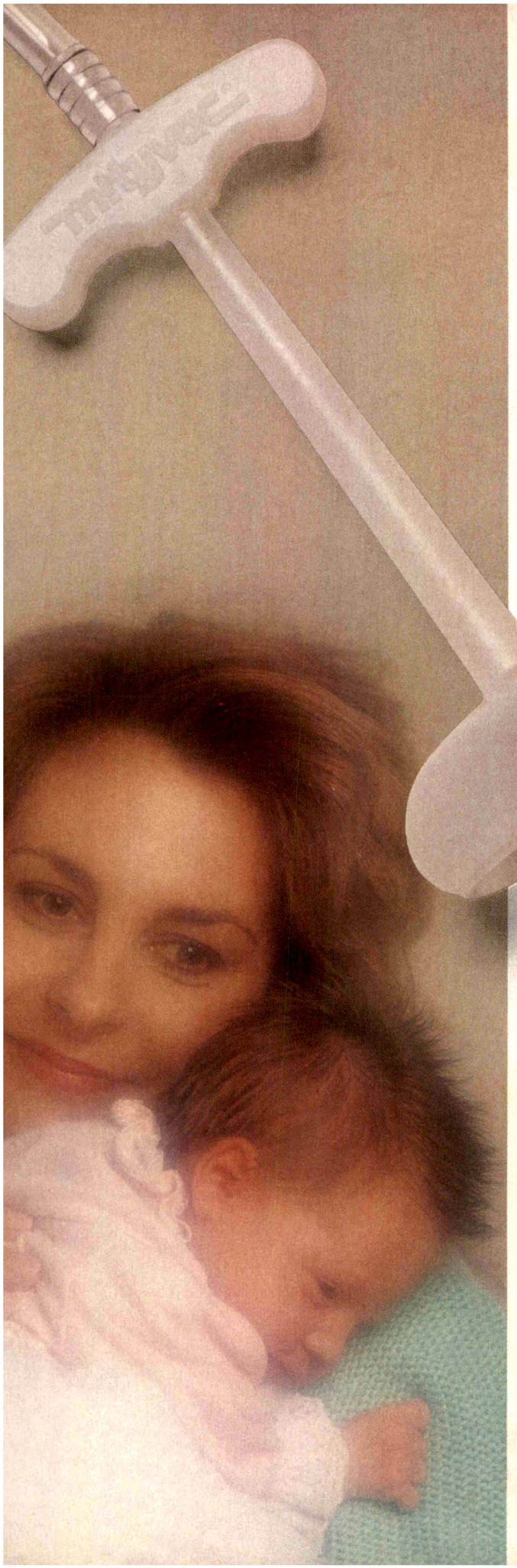
yet unidentified soluble immune cell factors may have contributed to embryo toxicity, especially in the 3 of 15 supernatants that retained toxic activity in spite of passage through the interferon gamma affinity columns.

The precise nature and extent of immunologic mechanisms for recurrent abortion are not well understood. The data presented in this study suggest that a potential mechanism of immunologic recurrent abortion may involve the secretion of inteferon gamma or other cytokines produced by activated lymphocytes and macrophages within female reproductive tissues in response to either trophoblast or sperm antigens.

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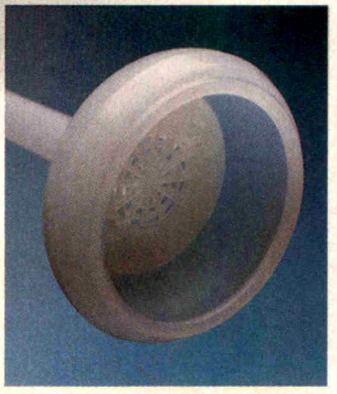


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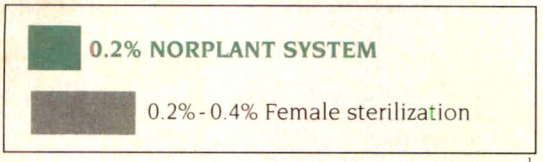


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Indications and Usage

The NORPLANT SYSTEM is indicated for the prevention of pregnancy and is a long-term (up to 5 years) reversible contraceptive system. The capsules should be removed by the end of the 5th year. New capsules may be inserted at that time if continuing contraceptive protection is desired.

Contraindications

1. Active thrombophlebitis or thromboembolic disorders. 2. Undiagnosed abnormal genital bleeding. 3. Known or suspected pregnancy. 4. Acute liver disease; benign or malignant liver tumors. 5. Known or suspected carcinoma of the breast.

Warnings

A. WARNINGS BASED ON EXPERIENCE WITH THE NORPLANT SYSTEM

1. *Bleeding Irregularities* — Most women can expect some variation in menstrual bleeding patterns. Irregular menstrual bleeding, intermenstrual spotting, prolonged episodes of bleeding and spotting, and amenorrhea may occur, and could mask symptoms of cervical or endometrial cancer. Overall, these irregularities diminish with continued use. Because amenorrhea may occur, missed menstrual periods cannot serve as the only identifier of early pregnancy. Perform pregnancy tests whenever pregnancy is suspected. If pregnancy occurs, the capsules must be removed.
Hemoglobin concentrations found in clinical trials generally indicated that reduced menstrual blood loss is associated with NORPLANT SYSTEM use. Blood loss resulting in hemoglobin values consistent with anemia occurred rarely.

2. *Delayed Follicular Atresia* — Atresia of the follicle is sometimes delayed, resulting in enlarged follicles that are clinically indistinguishable from ovarian cysts. In the majority of women, enlarged follicles disappear spontaneously. Rarely, they twist or rupture and surgical intervention may be required.

3. *Ectopic Pregnancies* — Ectopic pregnancies have occurred among NORPLANT SYSTEM users, although clinical studies have shown no increase in the rate of ectopic pregnancies per year among NORPLANT SYSTEM users as compared with users of no method or of IUDs. The incidence among NORPLANT SYSTEM users (1.3 per 1000 woman-years) was significantly below the rate estimated for noncontraceptive users in the U.S. (2.7 to 3.0 per 1000 woman-years). Ectopic pregnancy risk may increase with duration of NORPLANT SYSTEM use and increased weight of the user. Rule out ectopic pregnancy in any patient presenting with lower-abdominal pain.

4. *Breast-feeding* — Steroids are not the contraceptives of first choice for lactating women. Levonorgestrel has been identified in breast milk. Limited data suggests no significant effects on infant growth or health when mothers used the NORPLANT SYSTEM beginning 6 weeks after parturition.

5. *Foreign-body Carcinogenesis* — Rarely, cancers occur at foreign-body intrusion sites or old scars. None has been reported in NORPLANT SYSTEM clinical trials and risk to users is judged to be minimal.

6. *Thromboembolic Disorders* — Remove capsules if active thrombophlebitis or thromboembolic disease develops. With prolonged immobilization removal should be considered.

B. WARNINGS BASED ON EXPERIENCE WITH COMBINATION (PROGESTIN PLUS ESTROGEN) ORAL CONTRACEPTIVES (OCs)

Note: Many of the side effects or risks listed below are thought to be estrogen-related; the association of the NORPLANT SYSTEM progestin-only method to these risks is unknown.

1. *Cigarette Smoking* — Cigarette smoking increases the risk of serious cardiovascular side effects from combined OC use. Risk increases with age and heavy smoking (≥15 cigarettes/day) and is quite marked in women over 35 years old.

2. *Elevated Blood Pressure* — Increase in blood pressure has been reported in combination OC users; prevalence increases with long exposure.

3. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease is associated with combination OC use. Estimate of relative risk is 4- to 11-fold higher for users vs. nonusers.
Cerebrovascular Disorders: Combination OCs increase the relative and attributable risk of cerebrovascular events (thrombotic and hemorrhagic strokes). Generally, risk is greatest among hypertensive women > 35 years of age who smoke.
Myocardial Infarction (MI): An increased risk of MI has been attributed to combined OC use. This is thought to be primarily thrombotic in origin and related to the estrogen component. Increased risk occurs primarily in smokers or women with other underlying risk factors for coronary-artery disease. Relative risk of heart attack for combined OC users is estimated as 2 to 6 times that for nonusers. Absolute risk is very low for women under 30 years old.
Studies indicate a significant trend toward higher MI and stroke rates with increased progestin doses in combination OCs. However, recent data indicated no increased MI risk with past use of levonorgestrel-containing OCs.

4. *Carcinoma* — Recent evidence in the literature suggests no association between OC use and increased risk of breast cancer in the overall population of users. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on breast cancer risk for at least a decade following long-term use. Some of these same studies have shown an increased relative risk of breast cancer in certain subgroups; no consistent pattern has been identified. Some studies suggest an association between combination OCs and an increase in the risk of cervical intra-epithelial neoplasia in some populations of women. The extent to which such findings may be due to differences in sexual behavior and other factors remains controversial. A cause-and-effect relationship between combined OC use and breast or cervical cancer has not been established. Combination OCs may decrease ovarian and endometrial cancer risk. Irregular bleeding patterns associated with NORPLANT SYSTEM use could mask cervical or endometrial cancer symptoms.

5. *Hepatic Tumors* — Hepatic adenomas are associated with combination OC use; estimated incidence is 3 events per 100,000 users per year. Risk increases after 4 or more years of use. Hepatic adenomas are benign but may rupture and cause death through intra-abdominal hemorrhage.

6. *Ocular Lesions* — Retinal thrombosis is associated with OC use and is believed to be related to the estrogen component. However, NORPLANT SYSTEM capsules should be removed if there is unexplained partial or complete vision loss; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Undertake appropriate diagnostic and therapeutic measures immediately.

7. *Use Before or During Early Pregnancy* — Extensive epidemiological studies reveal no increased risk of birth defects when OCs are used prior to pregnancy. Studies also do not suggest a teratogenic effect when taken inadvertently during early pregnancy. No evidence suggests that risk with NORPLANT SYSTEM use is different.

8. *Gallbladder Disease* — Early studies reported an increased lifetime relative risk of gallbladder surgery in OC or estrogen users. More recent studies, however, indicate that the relative risk of gallbladder disease with OC use may be minimal; this may be related to use of OCs with less estrogen and progestin content.

Precautions

GENERAL

1. *Physical Examination and Follow-up* — A complete medical history and physical examination should be taken prior to implantation or reimplantation of NORPLANT SYSTEM capsules and at least annually during its use. Exams should include special reference to the implant site, blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. Rule out malignancy in cases of undiagnosed, persistent or recurrent abnormal vaginal bleeding. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. *Carbohydrate Metabolism* — Altered glucose tolerance is found in some combination and progestin-only OC users. Effects of NORPLANT SYSTEM on carbohydrate metabolism appear minimal. Observe diabetic and prediabetic patients carefully while using the NORPLANT SYSTEM. Follow women being treated for hyperlipidemias closely if using the NORPLANT SYSTEM. Some progestins may elevate LDL and may render control of hyperlipidemias more difficult. (See **Warnings**.)

3. *Liver Function* — Consider removing capsules if jaundice develops. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. *Fluid Retention* — Steroid contraceptives may cause some degree of fluid retention. Prescribe with caution, and careful monitoring, in patients with conditions possibly aggravated by fluid retention.

5. *Emotional Disorders* — Consider removing capsules if significant depression occurs since the symptom may be drug-related. Observe carefully those with history of depression and consider removal if depression recurs to a serious degree.

6. *Contact Lenses* — Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. *Insertion and Removal* — Insertion is advised during the first 7 days of the cycle or immediately following abortion to insure that the woman is not pregnant and to assure contraceptive effectiveness during first cycle of use. Capsules may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for the remainder of the cycle. Insertion is not recommended before 6 weeks postpartum in breast-feeding women. Follow insertion and removal instructions closely. Healthcare professionals are strongly advised to be instructed in the procedures before they attempt them. Proper insertion just under the skin facilitates removals; proper insertion and removal should result in minimal scarring. If all capsules cannot be removed at first attempt, attempt removal later when the site has healed. Bruising may occur at implant site during insertion or removal. Hyperpigmentation may occur over implant site but is usually reversible following removal. **See Full Prescribing Information for Detailed Insertion/Removal Instructions.**

8. *Infections* — Implant site infection has been uncommon (0.7%); aseptic technique and proper insertion/removal reduces possibility of infection. Institute treatment if infection occurs; remove capsules if infection persists.

9. *Expulsion* — Expulsion of capsules was uncommon; frequency increased when capsule placement was extremely shallow, was too close to incision, or when infection was present. Replace expelled capsule with new sterile capsule. Treat and cure any infection before replacement. Contraceptive efficacy may be inadequate with fewer than 6 capsules.

10. *Provisions for Removal* — Advise women that capsules may be removed at any time for any reason. Personnel instructed in removal technique should perform removal on request or at the end of 5 years of usage. Upon removal, dispose of capsules in accordance with Centers for Disease Control Guidelines for biohazardous waste.

DRUG INTERACTIONS: Reduced efficacy (pregnancy) in NORPLANT SYSTEM users has been reported when phenytoin or carbamazepine were used concomitantly. Warn NORPLANT SYSTEM users of possible decreased efficacy with use of related drugs.

DRUG/LABORATORY TEST INTERACTIONS: 1. Sex-hormone-binding globulin concentrations are decreased. 2. Thyroxine concentrations may be slightly decreased and triiodothyronine uptake increased.

CARCINOGENESIS: See **Warnings** section and Full Prescribing Information.

PREGNANCY: Pregnancy Category X. See **Warnings** section and Full Prescribing Information.

NURSING MOTHERS: See **Warnings** section and Full Prescribing Information.

INFORMATION FOR THE PATIENT: See Patient Labeling. Provide copy of patient labeling to the patient. Advise patients that Prescribing Information is available upon request. Inform prospective users of risks and benefits associated with NORPLANT SYSTEM use, with other forms of contraception, with no contraception, and about insertion/removal procedures. Informed consent from all patients may be desired in light of techniques involved with insertion and removal.

Adverse Reactions

The following have been associated with the NORPLANT SYSTEM during first year of use: many bleeding days or prolonged bleeding (27.6%); spotting (17.1%); amenorrhea (9.4%); irregular (onsets of) bleeding (7.6%); frequent bleeding onsets (7.0%); scanty bleeding (5.2%); pain or itching near implant site - usually transient - (3.7%); infection at implant site (0.7%); removal difficulties affecting subjects - based on 849 removals - (6.2%).
Controlled clinical studies suggest that the following, occurring during the first year, are probably associated with NORPLANT SYSTEM use: headache; nervousness; nausea; dizziness; adnexal enlargement; dermatitis; acne; change of appetite; mastalgia; weight gain; hirsutism, hypertrichosis, and scalp-hair loss. The following were reported with a frequency of 5% or greater during the first year and possibly may be related to NORPLANT SYSTEM use: breast discharge; cervicitis; musculoskeletal pain; abdominal discomfort; leukorrhea; vaginitis.

Overdosage

Overdosage may cause fluid retention with its associated effects and uterine bleeding irregularities.

Dosage and Administration

The NORPLANT SYSTEM consists of six Silastic® capsules, each containing 36 mg of the progestin, levonorgestrel. The total administered (implanted) dose is 216 mg. Implantation of all six capsules should be performed during the first 7 days of the onset of menses by a healthcare professional instructed in the NORPLANT SYSTEM insertion technique. Insertion is subdermal in the midportion of the upper arm about 8 to 10 cm above the elbow crease. Distribution should be in a fanlike pattern, about 15 degrees apart, for a total of 75 degrees. Proper insertion will facilitate later removal. **(See Full Prescribing Information for Detailed Insertion/Removal Instructions.)**

CI 4064-1 12/10/90

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Functional analysis of a mutant estrogen receptor isolated from T47Dco breast cancer cells

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OBJECTIVE: Estrogen receptor–positive cancers that initially respond to hormone therapy often progress to a resistant state. The breast cancer cell line T47Dco is a model for such resistance. It is a polymorphic line, composed of multiple cell populations that demonstrate the presence of mutant estrogen receptors by cloning and sequencing techniques. Our objective was to isolate and analyze the structural and functional characteristics of the T47Dco mutant estrogen receptor complementary deoxyribonucleic acid clones.

STUDY DESIGN: We constructed two independent T47Dco complementary deoxyribonucleic acid libraries. We isolated and sequenced T47Dco estrogen receptors and have identified a mutant receptor that is truncated near the end of the deoxyribonucleic acid binding domain. This mutant has now been recreated with site-directed mutagenesis and tested for its ability to bind to deoxyribonucleic acid, dimerize with other estrogen receptors, and activate gene transcription by the chloramphenicol acetyltransferase assay and by gel shift assays.

RESULTS: The chloramphenicol acetyltransferase assays reveal that in the absence of estradiol low levels of conversion of chloramphenicol to acetylated products occur when the mutant estrogen receptor is used to activate chloramphenicol acetyltransferase gene transcription, supporting that it has some constitutive function. Also, gel mobility shift assays demonstrate low levels of deoxyribonucleic acid binding with the mutant protein.

CONCLUSION: This mutant estrogen receptor may contribute to the estrogen receptor–positive, hormone-resistant phenotype of T47Dco cells by constitutively binding to and activating genes that were previously estradiol dependent. (Am J Obstet Gynecol 1992;166:1053-61.)

Key words: Breast cancer, hormone resistant, mutant estrogen receptors, deoxyribonucleic acid binding domain mutation, constitutive gene regulation

Estradiol stimulates normal mammary and endometrial epithelial growth and development in an action mediated by estrogen receptors. Estradiol also stimulates the growth of cancers that arise from these tissues in the early stages of the metaplastic process. The hormone binds to estrogen receptor, and the steroid-receptor complex interacts with chromatin to regulate specific gene transcription, bringing about estrogen-stimulated growth. This model is supported by studies that show a clear association between estrogen receptor levels and growth in human mammary and endometrial tumors,[1] by studies that show estrogen stimulation of cell growth in MCF-7 and ZR-75 human breast cancer cells[2, 3] and Ishikawa human endometrial cells,[4] and by studies in which estrogen-stimulated growth is suppressed by antiestrogens and rescued by estradiol.[5]

Tumors that require estrogen for growth are termed hormone dependent, and these tumors usually respond to endocrine therapy in the initial phases of treatment. However, as some tumors become anaplastic, their normal responses to estrogens are subverted; these tumors no longer require estradiol for growth and are classified as hormone resistant. Many resistant tumors no longer express estrogen receptors, and this may be the basis for their resistant state. However, 30% of all breast cancers are estrogen receptor positive, yet they do not respond to antiestrogen hormonal therapy.[6] The focus of our research has been to explain the estrogen receptor–positive, hormone-resistant phenotype. We hypothesize that genetic instability within some cancers leads to the generation of heterogeneous cell populations, some of which may express mutant estrogen receptors that function abnormally.

This model for hormone resistance is based on studies of the breast cancer cell line T47Dco. Previous studies from our laboratory have identified several unusual characteristics of this line.[7] By sucrose density gradient

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6/6/35622

analysis, T47Dco cells contain essentially no unfilled cytoplasmic (<3 fmol/mg protein) or nuclear estrogen receptors in the 8S region. Scatchard analysis of protamine sulfate–precipitated estrogen receptors shows that there are low levels of an unusual class of nuclear estrogen receptors present. They are unusual in that (1) they are tightly bound to chromatin without estrogen treatment and (2) the nuclear estrogen receptors can be measured only at elevated temperatures, suggesting a structural alteration in the protein.

In addition to the possibility that structural anomalies are present, the T47Dco estrogen receptors are functionally anomalous when compared with classically estrogen receptor–positive breast cancer cells such as MCF-7. Estradiol treatment fails to down-regulate the estrogen receptors of T47Dco cells, and antiestrogens do not suppress cell growth. Interestingly, however, T47Dco cells do express estrogen receptor messenger ribonucleic acid (mRNA). These cells also express very high levels of progesterone receptors, but the progesterone receptors of T47Dco cells cannot be regulated in the normal manner; that is, estradiol does not raise progesterone receptor levels and antiestrogens do not suppress them.

Like solid tumors, the T47Dco line exhibits genetic instability over time in cell culture. We have previously shown that the parental T47Dco line is composed of multiple cell populations, which, when cloned by limiting dilution, exhibit phenotypes varying from classically estradiol responsive to completely estradiol resistant.[8] On the basis of these observations, we postulate that T47Dco models stage IV breast cancer and that some of its subclones may express variant estrogen receptors.

To investigate this hypothesis, we previously constructed two independent complementary deoxyribonucleic acid (cDNA) libraries from the parental T47Dco line and cloned and sequenced several estrogen receptor cDNAs.[8] In addition to clones composed of normal estrogen receptor sequences, we identified several mRNAs that encode putative proteins with interesting mutations. We have expanded our investigations into the functional significance of one of these mutant receptors, the deoxyribonucleic acid (DNA) binding domain or DBD mutant, by using site-directed mutagenesis to generate an expression vector that has allowed us to express the protein in Cos-1 and HeLa cells. The ability of the mutant protein to bind to DNA, to form dimers with wild-type estrogen receptors, and to activate gene transcription is explored.

Material and methods

cDNA isolation and sequencing of mutant estrogen receptor cDNA. Two cDNA libraries were made. For library 1 polyadenylated mRNA, isolated from estrogen receptor–positive, estrogen-resistant T47Dco cells, was used to direct cDNA synthesis with two human estrogen receptor–specific oligonucleotides as primers. One 24-mer, 5′-CCC CAC TTC ACC CCT GCC CTC CCC-3′, was complementary to base pairs 1052 to 1075 of the estrogen receptor message in the hinge region D; the second, a 30-mer, 5′-GGG GGA AAG AGA GCT GTT ACA AAG ATT TAG-3′, was complementary to base pairs 2230 to 2259 in the 3′ untranslated region.[9-11] The cDNA was synthesized and cloned ino bacteriophage SWAJ-4 by Clontech (Palo Alto, Calif.).

In library 2 cytoplasmic polyadenylated mRNA was the starting material. Double-stranded cDNA was synthesized with the two estrogen receptor oligonucleotides used as primers. *Eco*RI/*Not*I linker-adapters were ligated to blunt-ended cDNA. The products were phosphorylated, and fragments larger than 300 bp were gel purified, electroeluted, and ligated to λ-gt10 cut with *Eco*RI and then dephosphorylated. Ligated recombinants were packaged into phage with *in vitro* packaging extracts (Invitrogen, San Diego) and were amplified in *Escherichia coli* C600hfl. The two cDNA libraries consisted of 4×10^5 and 3×10^6 independent recombinants before the amplification step.

Approximately 5×10^5 phage from each library were screened for estrogen receptor sequences by plaque hybridization with a 2.1 kb human estrogen receptor nick-translated cDNA probe encompassing the protein-coding region, which was kindly supplied by A. Baker.[11] Seven independently isolated types of recombinant phage from library 1 and five from library 2 were randomly picked and purified to homogeneity by three rounds of screening. Bacteriophage was prepared from plate lysates, and phage DNA was purified with Lambda Sorb (Promega, Madison, Wis.) according to the manufacturer's instructions.

For library 1 pGEM-1 containing the cDNA was prepared by recircularization after release from SWAJ-4 by restriction digestion with *Spe*I. For library 2 the cDNA insert was isolated with *Eco*RI and recloned into pGEM7Zf(+) (Promega). Double-stranded plasmid DNA containing the cDNA inserts was alkali denatured and both strands were sequenced (Sequenase, United States Biochemical Corp., Cleveland) with use of SP6 and T7 promotor primers and overlapping synthetic estrogen receptor oligonucleotide primers. Sequences were compared against those reported in the Genetic Sequence Data Bank (GenBank, Beckman, release 59, Palo Alto, Calif.).

Constructions of the DBD estrogen receptor mutant. Site-directed mutagenesis[10] was used to construct a pSG5 vector[12] containing the coding region for the DBD estrogen receptor mutant. Double-stranded wild-type human estrogen receptor in the expression vector pSG5 (HEGO-pSG5) was kindly provided by P. Cham-

bon and was transfected into TG2 *E. coli* with helper phage M13K07 (Promega). When growth with this phage occurred, single-stranded wild-type estrogen receptor template DNA was obtained. An oligonucleotide spanning the estrogen receptor sequence that was mutated in the DBD mutant was constructed on a Cyclone Plus DNA synthesizer (Milligen/Biosearch, Burlington, Mass.). Two thymidine residues were added to the sequence of the oligonucleotide to create the identical frame shift mutation that was present in the naturally occurring DBD estrogen receptor mutant from T47Dco, thus creating the TGA stop codon. Downstream from this site, an *Eco*RI restriction site was also added to the oligonucleotide sequence. This site was used to screen recombinants for the mutant sequence. The phosphorylated mutant oligonucleoide was annealed to the single-stranded wild-type template, and the second strand was elongated. The resultant double-stranded hybrid mutant-wild-type vector was used transform DH5 *E. coli* cells that were screened with *Eco*RI restriction digestion. Colonies expressing the mutant vector were isolated and purified by retransformation and repeat screening. The presence of the correct mutant seqence was verified by Sanger dideoxy sequencing.

The chloramphenicol acetyltransferase assay. The vit-tk-CAT reporter vector was constructed by placing the *Xenopus vitellogenin* A2 (vit) upstream sequence (−331 to −87) and the herpes simplex virus thymidine kinase (tk) promoter (−105 to +51) upstream from the bacterial gene for chloramphenicol acetyltransferase.[13] Chloramphenicol acetyltransferase enzyme assays were performed as described by Gorman et al.[14] Briefly, subconfluent HeLa and Cos-1 cells in 90 mm culture plates were cotransfected in duplicate by calcium phosphate coprecipitation with different amounts of the receptor expression vectors (DBD or wild-type), 3 μg of the β-galactosidase vector pCH110 (Clontech) and 1 μg of the estradiol-responsive reporter vit-tk-CAT. The total amount of DNA transfected was normalized to 20 μg per plate with the DNA vector Bluescribe M13+. The cells were cultured for 48 hours in the presence of 10^{-8} mol/L estradiol, and the cell extracts were prepared by four cycles of freeze-thawing followed by centrifugation at 10,000*g* for 15 minutes. The efficiency of transcription was determined by assaying each culture plate for β-galactosidase activity with spectrophotometric analysis. The chloramphenicol acetyltransferase analysis was performed with cell extract amounts equal to 50 (for HeLa cells) and 1000 (for Cos-1 cells) units of β-galactosidase activity for 1 hour at 37° C. The acetylated and nonacetylated forms of carbon 14–chloramphenicol were separated by thin-layer chromatography and quantitated from an autoradiograph of the thin-layer chromatography plate by densitometry. Substrate conversion was further quantitated by assessing the radioactivity present on the thin-layer chromatography plate in a scintillation counter.

Gel shift assay. HeLa and Cos-1 cells at 50% confluence in 90 mm culture plates were transiently transfected with 5 μg of plasmid DNA of receptor expression vector (wild-type or DBD mutant estrogen receptor) and 3 μg of pCH110 vector and then normalized for DNA transfection to 20 μg with Bluescribe M13+ by the calcium phosphate coprecipitation technique. The cells were grown for 48 hours in the absence of hormone, and then 10^{-8} mol/L estradiol was added to the culture media 2 hours before harvesting. The cells were washed with chilled phosphate-buffered saline solution, collected, and homogenized in 20 mmol/L Tris-hydrochloride (pH 7.5), 2 mmol/L dithiothreitol, and 20% glycerol containing 0.4 mol/L potassium chloride. After centrifugation at 100,000*g* for 1 hour at 4° C, the whole-cell-extract supernatant was collected and transcriptional efficiency was determined by β-galactosidase activity. The gel shift experiments were carried out after the method of Kumar and Chambon.[15] Briefly, whole-cell extract containing either individual receptor forms or a mixture of two forms (8 to 12 μg protein) was incubated with 2 μg of poly (dI:dC) (Pharmacia, Piscataway, N.J.) at 0° C for 15 minutes. The binding reaction, initiated by adding 35,000 counts/min per lane of phosphorus 32-5′ end-labeled oligonucleotide probe representing the wild-type estrogen response element corresponding to −308 to −342 of the promoter-upstream element of the *X. vitellogenin* A2 gene, was incubated at 20° C for 20 minutes. Some reactions were also incubated in the presence of 100 ng of unlabeled estrogen response element DNA, 100 ng of unlabeled progesterone response element DNA, or estrogen receptor antibody (H-222 or H-226, Abbott, Chicago). The reactions were loaded onto a prerun 4.5% polyacrylamide gel containing ½× TBE (25 mmol/L Tris, 25 mmol/L boric acid, 0.5 mmol/L ethylenediaminetetraacetic acid) buffer and run at 20 mA for 1.5 to 2 hours. The gel was then vacuum dried for autoradiography.

Results

Naturally occurring estrogen receptor mutations in T47Dco cDNA. Two independent cDNA libraries were constructed from the parental T47D line, T47Dco. Library 1 was made from total polyadenylated mRNA and yielded clones with the wild-type estrogen receptor sequence and several clones with mutant sequences. One mutant clone from this library was 0.9 kb in length, encoding part of exon 2 and all of exon 3. The mutant sequence has an insertion of two thymidine residues that changes amino acid 250 from methionine to isoleucine when compared with the wild-type sequence. The TT insertion results in a frame shift and the stop codon TGA is generated. Thus the protein resulting

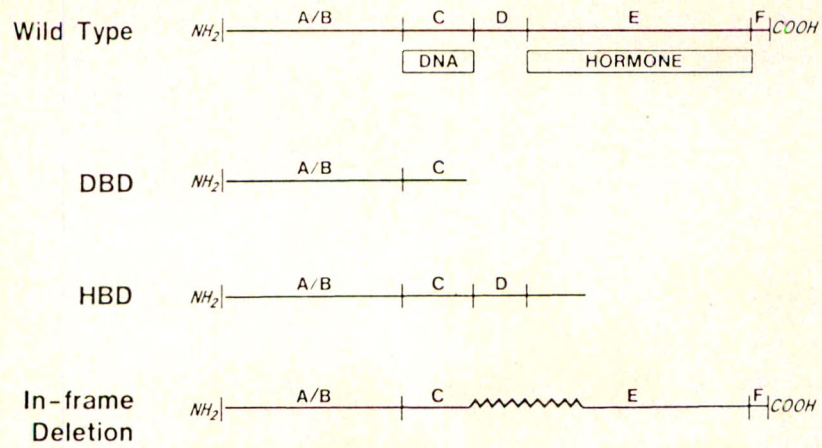


Fig. 1. Estrogen receptor forms isolated from T47Dco. Schematic representation of functional domains in wild-type estrogen receptor and the proteins encoded by mutant estrogen receptor cDNA of T47Dco cells. Functional regions of wild-type estrogen receptor (595 amino acids) include amino terminus domain *A/B*, DNA binding domain *C*, hinge region *D*, hormone binding domain *E*, and carboxy terminus *F*. Predicted structures of mutant proteins are also shown including DBD mutant truncated at amino acid 250, HBD mutant truncated at amino acid 417, and 153 amino acid in-frame deletion *(jagged line)* mutant.

from the translation of this message would be truncated after amino acid 250 in the DNA binding domain (domain C, DBD), just beyond the last cysteine (amino acid 245) of the second DNA-binding finger, and is designated the DBD mutant.

Library 2 also yielded clones with normal estrogen receptor sequences, as well as two types of mutant clones that are described here. The first mutant cloned from this library has undergone a single deletion of a guanine residue at amino acid 411 in the hormone binding domain of the receptor. The loss of this single base results in a frame shift and the generation of the stop codon TAG seven amino acids downstream from the mutation. Thus the protein encoded by this message would have an intact DNA binding domain and a normal hinge region (domain D); however, the hormone-binding domain would be truncated at amino acid 417. The last seven amino acids of the protein located downstream from the deletion would be novel and would not be present in the wild-type sequence. This clone has been designated the HBD mutant. The second library also yielded two independent clones of 820 and 980 nucleotides that have the identical deletion of 462 bp. The deletion occurs in-frame with the codons for the amino acids, and no frame shift occurs. These clones contain a wild-type upstream sequence from the 5′ untranslated region through the first four codons of exon 4. At this point the sequence is missing the next 462 nucleotides, including most of exons 4 and 5. The last codon of exon 5 is preserved, as are sequences in exon 6. These two independent clones would encode a 442-amino-acid mutant estrogen receptor protein instead of the native 595-amino-acid protein. The deletion includes part of the DNA binding domain, the entire hinge region, and a portion of the hormone-binding domain. This mutant has been designated the in-frame deletion. The T47Dco mutant cDNA clones have been reported previously,[8] and are shown schematically in Fig. 1 for reference.

Generation of a DBD mutant estrogen receptor expression vector and functional analysis of the mutant protein. We used site-directed mutagenesis to construct a pSG5 expression vector that contains the DBD mutant estrogen receptor sequence. The wild-type estrogen receptor pSG5 vector (HEGO-pSG5) was kindly provided by P. Chambon. The sequences of the wild-type and the DBD mutant estrogen receptor coding regions within the vector were verified by DNA sequencing (Fig. 2). These vectors were then transiently transfected into Cos-1 and HeLa cells by the calcium phosphate coprecipitation method. The cells were harvested and the whole-cell extracts were used to perform chloramphenicol acetyltransferase and gel mobility shift assays.

Fig. 3 shows the results of an assay testing the abilities of wild-type estrogen receptor and DBD mutant estrogen receptor to activate chloramphenicol acetyltransferase gene transcription. When the wild-type was transfected alone (lanes 1 and 2), conversion of ^{14}C-chloramphenicol to the acetylated products occurred in amounts significantly greater than the baseline conversion that occurred when the vit-tk-CAT vector was transfected alone (lanes 9 and 10). The DBD mutant transfection is seen in lanes 3 and 4. Chloramphenicol acetyltransferase activity by the DBD mutant was 7% increased over the baseline activity of the host cell in

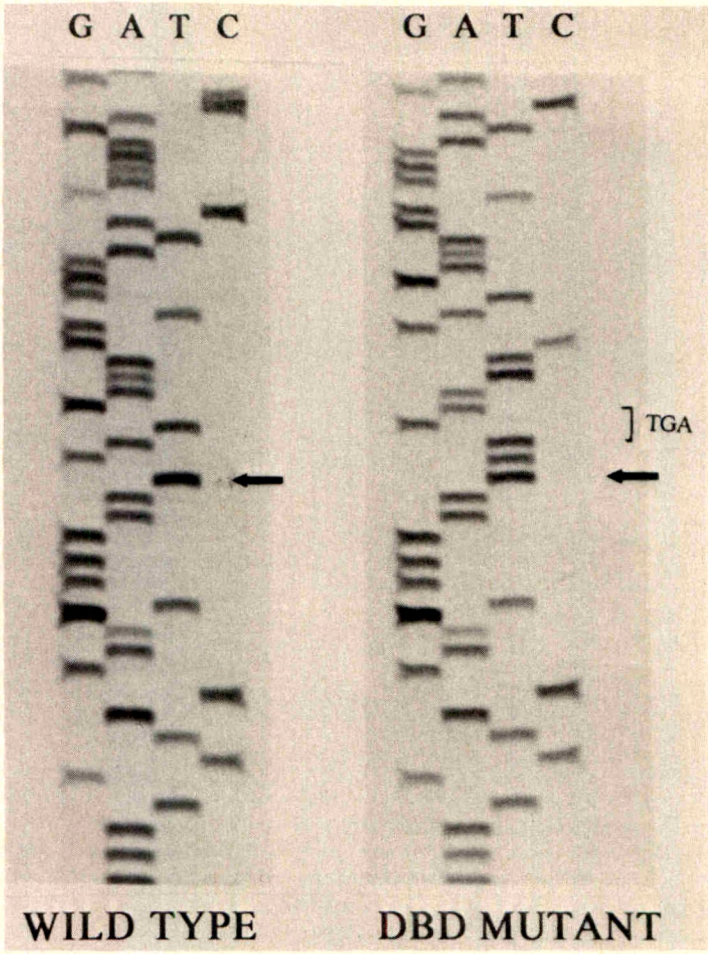


Fig. 2. Sequencing gel of wild-type and DBD mutant vectors. This figure provides proof of construction of DBD mutant. Wild-type estrogen receptor sequence is on left and DBD mutant is on right. *Arrow*, Compare with wild-type sequence. We successfully inserted two thymidine residues into DBD mutant sequence, like those originally found in naturally occurring clone. Two thymidine bases inserted generated frame shift and stop codon, TGA, which is also indicated here.

the absence of estrogen receptor. This finding was confirmed by densitometry measurements of the autoradiograph and by counting the ^{14}C-substrate and product from the thin-layer chromatography plate in a scintillation counter. Additionally, cotransfecting the DBD mutant and wild-type into the same cells (lanes 5, 6, 7, and 8) resulted in chloramphenicol acetyltransferase activity that may be slightly depressed compared with the activity of the wild-type alone. Thus, at the concentrations of DNA tested, the mutant appears to be capable of weakly activating gene transcription; also at very high concentrations ($\times 10$) it can slightly decrease wild-type gene activation.

To test the ability of the DBD mutant to bind to DNA, we used gel mobility shift experiments with a ^{32}P-labeled estrogen response element oligonucleotide.[15] Whole-cell extracts from cells transfected with wild-type or DBD receptor vectors were isolated and allowed to bind to the DNA. These receptor-DNA complexes were run on a polyacrylamide gel. If the extract contained functional estrogen receptors, the shifted bands that resulted from estrogen receptor binding to the estrogen response element were detected on an autoradiograph of the gel, and their estrogen receptor specificity was verified by antibody supershift.

Our experiments indicate that wild-type estrogen receptor binds strongly to the estrogen response element, resulting in three specific shifted bands. Several other nonspecific bands that result from protein-DNA interactions that are independent of estrogen receptor are also seen. The estrogen response element binding specificity of the protein-DNA complexes is confirmed by the fact that they can be competed by preincubating the cell extract with 100-fold excess of unlabeled estrogen response element, but are not competed by preincubation with 100-fold excess of the progesterone response element. The estrogen response specificity of the major band is confirmed by the fact that it is up-

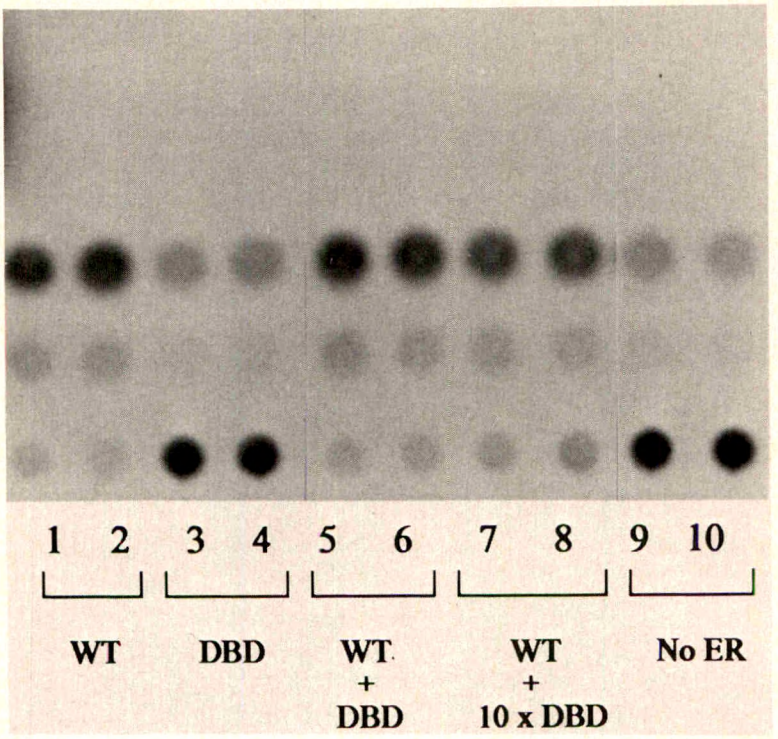


Fig. 3. Transcriptional analysis of wild-type and DBD mutant estrogen receptor. In chloramphenicol acetyltransferase assay substrate, ^{14}C-labeled chloramphenicol, is converted to its acetylated products only if enzyme chloramphenicol acetyltransferase is produced. Amount of product formed is a measure of chloramphenicol acetyltransferase gene transcription under control of estrogen receptor *(ER)*. Last two lanes of this gel show that little conversion of substrate to product occurs when estrogen response element–chloramphenicol acetyltransferase vector is transfected alone into Cos-1 cells, without estrogen receptor. We then compared choramphenicol acetyltransferace activity with that of wild-type *(WT)* estrogen receptor alone, as seen in *lanes 1 and 2;* with DBD mutant alone, *lanes 3 and 4;* and with cotransfection of both mutant and wild-type vertors, *lanes 5 to 8.*

shifted when the estrogen receptor antibodies H-222 and H-226 are added to the reaction mixture. H-222 recognizes an epitope in the hormone binding domain, while H-226 recognizes the DNA binding domain upstream from the site of the DBD truncation.[10]

Our results also confirm that little estrogen response element binding occurs when the DBD mutant is transfected alone. However, the addition of H-226 to the whole-cell extract and estrogen response element mixture does result in a faint, upshifted band that has the identical electrophoretic mobility as the wild-type H-222 upshifted band. Note that the epitope for H-222 would theoretically be missing in the DBD mutant. The significance of this band and whether it truly represents a supershifted estrogen receptor and estrogen response element complex that has resulted from DBD DNA binding remain unclear until expressed protein levels can be accurately quantitated. Cotransfecting the DBD mutant with wild-type estrogen receptors resulted in estrogen response element binding that appeared to be identical to that by wild-type estrogen receptors alone. However, a heterodimer would be difficult to detect under these conditions of extensive wild-type DNA binding. Note that in lane 14, with no transfected estrogen receptors, a nonspecific band migrated in a position similar to that of the major specific estrogen receptor band. This can also be seen in lanes 3, 7, and 12 to which cold specific estrogen response element DNA has been added, indicating that this band is nonspecific. Adding H-222 estrogen receptor antibody to the lane without estrogen receptor does not result in a supershifted band (data not shown).

Comment

The molecular biologic characteristics of the estrogen receptor have been extensively explored in recent years. The cDNA was independently cloned and sequenced from MCF-7 breast cancer cells by two groups in 1986, Green et al.[9] and Greene et al.[11] The protein is encoded by a 6322-nucleotide mRNA. On the basis of this work, Kumar et al. distinguished six functional domains of the receptor.[10, 15] The domains are identified by the letters A through F. The C domain contains two DNA-binding zinc fingers and is the region of the pro-

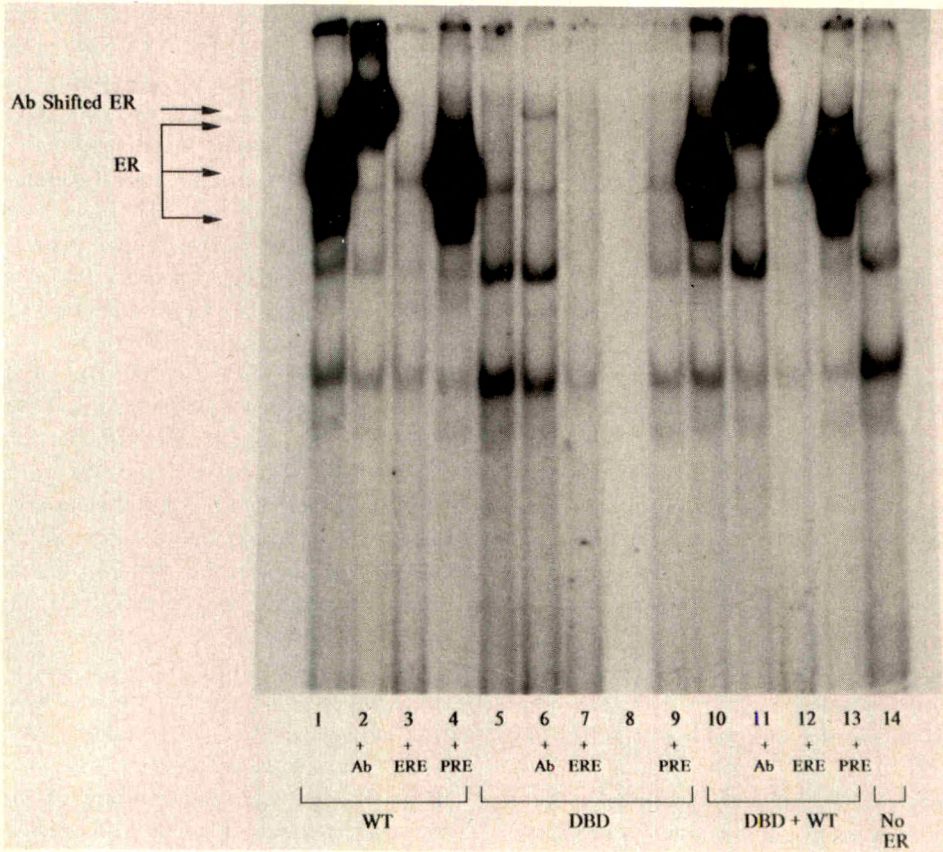


Fig. 4. Gel mobility shift assay of wild-type *(WT)* and DBD mutant estrogen receptor *(ER)* binding to estrogen response element *(ERE)*. *Lane 1* has shifted bands that result from interaction of HeLa whole-cell extract containing wild-type estrogen receptor with estrogen response element DNA. *Lane 2* shows supershifted band that has resulted from addition of estrogen receptor antibody H-222. Some nonspecific shifted bands that have resulted from protein-DNA interactions that are not related to estrogen receptor are also shown. *Lane 3* shows that specific estrogen receptor–shifted band can be competed out by addition of 100-fold excess of unlabeled specific estrogen response element DNA. Unlabeled estrogen response element DNA has displaced estrogen receptor from labeled estrogen response element; however, nonspecific shifted bands are still present. In *lane 4* 100-fold excess of unlabeled nonspecific DNA has been added. In this case progesterone response element *(PRE)* has been used, which does not bind estrogen receptor. *Lanes 5, 6, 7, and 9* represent identical experiment with DBD mutant protein and H-226 anti–estrogen receptor antibody. *Lane 8* is blank. *Lanes 10 to 13* represent estrogen receptor binding when wild-type and DBD mutant are cotransfected into same cells. In *lane 14* cells have been transfected with expression vector lacking the estrogen receptor cDNA insert.

tein that binds to the estrogen response element. Mutations in this portion of the protein were shown to change the affinity of the protein for DNA, resulting in suboptimal or complete loss of DNA binding. The hormone binding capability of the receptor resides in region E, and mutations in this region affect the ability of the receptor to bind ligand. Because these two functions, DNA binding and hormone binding, are carried out by separate parts of the protein, they are to some extent independent. Thus it appears to be possible to have variant receptors that can bind to DNA with limited affinity without first binding hormone and vice versa.[10]

Reports of naturally occurring mutant or variant estrogen receptor forms have recently appeared in the literature. Several polymorphic forms of the estrogen receptor gene have been described.[16-18] The majority of these genetic changes have occurred in introns, which do not directly encode the mRNA or, in turn, the protein. However, several exon mutations have also been found. Garcia et al.[19] identified a mutation in the B region of estrogen receptor mRNA in some human breast cancer biopsy specimens that represents a polymorphic variant estrogen receptor form. This mutation has since been correlated with lower than normal levels of hormone binding.[20] Preliminary evidence suggests that women who are heterozygous for this mutation

have a higher proportion of spontaneous abortions than those who are homozygous at the same locus.[21]

Estrogen receptor mRNAs from several tissues and species are reported to be the same size: 6.3 kb.[22] However, Murphy and Dotzlaw[23] have identified smaller-sized estrogen receptor–like mRNAs in human breast cancer biopsy samples by Northern blotting. Some of these smaller-size messages appear to lack significant portions of the 3′ region of the message, including the hormone binding domain. Fuqua et al.[24] have also identified a mutant form of estrogen receptor that is missing part of the hormone binding domain (exon 5) by polymerase chain reaction amplification of mRNA from tumor specimens. This mutant is an alternatively spliced form that is capable of constitutively activating transcription of a normally estrogen-dependent gene construct in yeast cells. Polymerase chain reaction amplification was also used to identify a mutation in the D domain of estrogen receptor mRNA expressed in a murine transformed Leydig cell line, B-1 F.[25] The functional significance of these mutations has yet to be fully explored.

Our investigations of the T47Dco breast cancer line have determined that T47Dco expresses mutant estrogen receptor mRNA that may encode anomalous proteins. We are interested in the functional significance of these putative proteins. Do these mutants explain the heterogeneity and estradiol-resistant phenotype of the parental cell line? Can the mutants bind to DNA? Do they dimerize with other estrogen receptors? Can they activate gene transcription?

To begin to address these questions, we are constructing the mutant estrogen receptors by site-directed mutagenesis. Preliminary data for the DBD mutant are described here. We have shown that this protein has some capacity to bind to DNA and to activate gene transcription with gene mobility shift (Fig. 4) and chloramphenicol acetyltransferase assays (Fig. 3). Furthermore, in cotransfection experiments, the DBD mutant may not significantly interfere with the ability of the wild-type receptor to bind to DNA on gel shift, although it slightly decreases chloramphenicol acetyltransferase activity. Additionally, the lack of an intermediate-sized shifted band in cotransfected cells (Fig. 4) suggests that no heterodimers form between the DBD mutant and wild-type receptor. Considering the structure of the mutant, these results are not surprising.

On the basis of an analysis of the domains of the wild-type estrogen receptor and a series of extensively tested truncation mutants constructed by Kumar et al.[10, 15] the functional capabilities of the DBD mutant can be predicted. This protein is truncated after amino acid 250 within the DNA binding domain; as a result, its DNA binding capabilities are expected to be greatly diminished. The entire hormone binding domain is missing, which effectively precludes ligand binding. Thus the very low level of DNA binding that may occur, as evidenced by the faint supershifted band shown in Fig. 4 in the presence of the estrogen receptor antibody H-226, must be hormone independent.

What would be the theoretical consequences of the expression of such a protein? Cells may demonstrate a chronic; low level of constitutive expression of estrogen-dependent genes; that is, estrogenic responses would be up-regulated in the absence of estradiol—a classic estrogen-resistant scenario.

Theoretically, a mutant like the DBD protein can squelch the normal estrogen receptor even if it has no capacity to stimulate transcription as a homodimer or to form heterodimers with wild-type estrogen receptor. The mutant may bind up transcription factors that are needed by the functional receptor and thereby limit its normal transcriptional activity. Squelching is a subset of a dominant-negative activity that may lead to an estrogen-resistant state.

We have shown that T47Dco breast cancer cells express mutant estrogen receptor mRNAs that may encode abnormal estrogen receptor proteins. In additional experiments we have reconstructed one of these naturally occurring mutants, the DBD estrogen receptor and have begun to analyze its functional characteristics. Future experiments will address the activity of the HBD and the in-frame deletion mutants in an attempt to understand and account for the estrogen-resistant T47Dco phenotype. Our data, along with the work of others, support the possibility that functional and nonfunctional estrogen receptor mutant proteins may be present in some cells. The significance of these findings with respect to the prognosis and treatment of breast and endometrial cancer and as well as the possible role of mutant estrogen receptors in the pathogenesis of other obstetric and gynecologic problems, such as recurrent pregnancy loss, await further elucidation.

We thank Drs. Pierre Chambon and Andrew Baker for sending us the necessary vectors. Drs. Lin Tung, Glenn Takimoto, Nancy Krett, Mark Graham, Bill Wood, David Gordon, and Louise Miller have made suggestions and provided us with assistance during this work. Kate Strand provided technical support. Abbott Laboratories kindly provided antibodies H-222 and H-226.

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Laser surgery–operative laparoscopy: Technology assessment

Laser surgery–operative laparoscopy panel

Introduction

It has become apparent that innovations in medicine are frequently expensive, inadequately studied, aggressively promoted, and, perhaps, prematurely incorporated into clinical practice. Two years ago the American Gynecological and Obstetrical Society decided to sponsor each year at this meeting a thorough critique of a new or controversial test, procedure, or treatment. The participants are chosen from the membership of the Society as individuals who will objectively evaluate the literature and who have no personal or vested interest in the outcome or conclusion.

Frontiers of operative laparoscopy: A review and critique of the evidence

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OBJECTIVES: This report reviews the evidence supporting the use of operative laparoscopy for a number of expanded indications in gynecology.
STUDY DESIGN: Studies published in English were identified through a Medline search back to 1966 for literature listed under the medical subject heading of "peritoneoscopy"; this search was supplemented by reviews of reference lists and discussions with experts.
RESULTS: Fair evidence exists to recommend use of laparoscopy for ectopic pregnancy, ovarian biopsy, and treatment of polycystic ovarian syndrome resistant to clomiphene citrate therapy. Evidence concerning other indications is inadequate to allow recommendations.
CONCLUSION: Mechanisms are urgently needed to evaluate surgical innovations in gynecology with the same degree of scientific rigor currently afforded medical innovations. Without surgical technology assessment, the current double standards in gynecologic therapy will persist. (Am J Obstet Gynecol 1992;166:1062-71.)

Key words: Laparoscopy, peritoneoscopy, ectopic pregnancy

Imagination and daring must be coupled with a healthy coefficient of skepticism.[1]

In recent years the uses for the laparoscope in gynecology have expanded dramatically from diagnosis and tubal sterilization to treatment of ectopic pregnancy and ovarian neoplasms. The 1988 membership survey of the American Association of Gynecologic Laparoscopists[2] provides an index of the frequency of these newer uses. Although only 24% of the questionnaires were returned with usable responses, 880 physicians reported having performed 36,928 newer procedures in that year (Fig. 1), including >5000 cases involving ovarian cysts and >4000 procedures to destroy the uterosacral nerves.

However, the safety and efficacy of these expanded uses for operative laparoscopy compared with that of laparotomy remain largely unknown. In the Association's survey[2] the reported serious complication rate was 15 per 1000 procedures; the mortality rate was 5 per 100,000 procedures. On the basis of limited information, some enthusiasts have proclaimed that "The obituary of laparotomy for pelvic reconstructive surgery has been written . . .,"[3] while others have cautioned that "To depict a 4-hour endoscopic marathon as prog-

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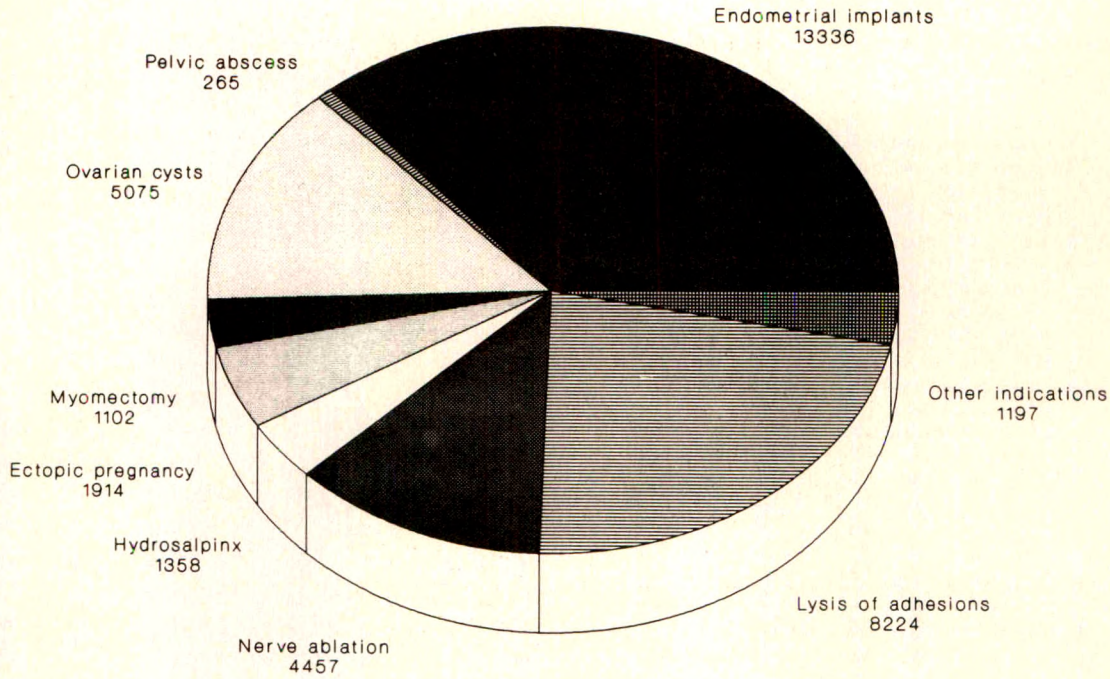


Fig. 1. Number of operative laparoscopies by indication performed by 880 members of the American Association of Gynecologic Laparoscopists (1988).[2]

ress when the same result could have been achieved by laparotomy within 2 hours is ludicrous."[4] The field of operative laparoscopy is now populated by both "kamikaze surgeons who will push the bounds of this surgery to the outer limits" and physicians who "remain as maladroit as a beetle on its back and should stay with conventional techniques."[4]

Operative laparoscopy has several potential advantages over conventional laparotomy. Operating times may be shorter for some procedures, and postoperative recovery is faster.[5] Adhesion formation may be less likely.[6] Shorter hospital stays may translate into substantial economic advantages.[7] As pointed out in a thoughtful review, "The crucial question, however, is whether the results are equivalent to those of laparotomy."[8] My article attempts to address this question by evaluating the literature on some emerging roles for operative laparoscopy.

Material and methods

This review included management of ectopic pregnancy, ovarian biopsy, treatment of ovarian cysts, adnexectomy, treatment of polycystic ovary syndrome, management of pelvic abscess, uterosacral nerve ablation, ventrosuspension of the uterus, and hysterectomy. Laparoscopic management of endometriosis or infertility and treatment with intraabdominal lasers (covered elsewhere in this symposium) were excluded from consideration.

To identify relevant publications I conducted a Medline search back to 1966 for literature listed under the medical subject heading of "peritoneoscopy." I also consulted reference lists in textbooks as well as those of articles retrieved through the Medline search. These were supplemented by reviews of syllabus references from Association-sponsored courses and discussions with experts in the field. My review was restricted to reports in English. Although a number of papers have been published in French and German, most of them appeared to be descriptive studies similar to those published in English.

I categorized each report by type of study, grouped reports by indication for operative laparoscopy, and evaluated the quality of the evidence supporting the use of laparoscopy for that indication. For this evaluation I adapted the United States Preventive Services Task Force rating scale[9] (Table I), which was modified from a method developed by the Canadian Task Force on the Periodic Health Examination.[10] This hierarchy of studies gives greater weight to those studies that are, in general, less subject to bias and misinterpretation. Randomized controlled trials were accorded the greatest weight, cohort and case-control studies (analytic studies) or comparisons between time and places intermediate weight, and case-series and case reports (descriptive studies) or expert opinions the least weight.

In addition to categorizing study types, I also evaluated the quality of the research methods. Because none of the published randomized controlled trials could be considered to have been properly designed,[9] each study was considered class II evidence. Similarly, unless cohort and case-control studies were well

Table I. Quality of evidence

I	Evidence obtained from at least one properly designed randomized controlled trial.
II-1	Evidence obtained from well-designed controlled trials without randomization.
II-2	Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group.
II-3	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.
III	Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Source: United States Preventive Services Task Force.

Table II. Strength of recommendations

A	There is good evidence to support the recommendation that operative laparoscopy be used for this indication.
B	There is fair evidence to support the recommendation that operative laparoscopy be used for this indication.
C	There is inadequate evidence regarding the use of operative laparoscopy, for this indication, but recommendations may be made on other grounds.
D	There is fair evidence to support the recommendation that operative laparoscopy not be used for this indication.
E	There is good evidence to support the recommendation that operative laparoscopy not be used for this indication.

Source: United States Preventive Services Task Force.

designed[9] they were considered class III evidence. Case-series reports, the most frequent study design, were judged class III evidence. This modification of the classification scheme[9] was used to incorporate both the type and quality of evidence available for making clinical recommendations.

Using a five-point rating scale (Table II), I assessed the strength of the recommendation that can be made concerning each use of operative laparoscopy based on the existing evidence. This scale was modified from that used by the United States Preventive Services Task Force.[9] Indications for which laparoscopy has been proved safe and effective in well-designed studies (class A) were recommended. Similarly, uses for which laparoscopy has consistently been shown to be safe and effective in a large number of studies of lesser merit (class B) were recommended. Those uses that have been shown to be unsafe or ineffective (classes D and E) were not recommended. When evidence was insufficient in quality or quantity to make a recommendation (class C), none was made.

Results

Ectopic pregnancy. Ectopic pregnancy has been more thoroughly studied than other expanded indications for operative laparoscopy (Table III). Two groups of investigators have evaluated management of ectopic pregnancy in randomized controlled trials. In a study of 60 patients with unruptured tubal pregnancies, Vermesh et al.[11] randomized half to undergo salpingostomy by laparoscopy and half by laparotomy. Three patients (10 per 100 procedures) in each group sustained complications; the group having laparoscopy had significantly shorter hospital stays and recuperations. However, as noted by the authors, the power of this trial to detect important differences in outcomes was very low. To show that laparoscopy was associated with a 50% reduction in morbidity (α 0.05, power 80%, and equal numbers of subjects in both groups), 948 patients would be required.

In a similar study by Lundorff et al.,[6] 73 patients underwent a second laparoscopy to evaluate adhesion formation after treatment for ectopic pregnancy. In the group having had prior laparoscopic treatment, the ipsilateral tube had adhesions less often (42 per 100) than in the group having had laparotomy (76 per 100; relative risk 0.5; 95% confidence interval 0.3 to 1.0). The rates of tubal patency were not significantly different. The comparison was biased because the two groups had different operations performed on the tubes. Moreover, 30% of subjects initially randomized were not evaluated and rates of follow-up were substantially different for the two treatment groups, raising the possibility of ascertainment bias.

Two groups of investigators compared laparoscopy with laparotomy in cohort studies. Brumsted et al.[12] performed a retrospective cohort study that they incorrectly characterized as a case-control study. The rate of operative complications was identical in the two groups (8 per 100 procedures), although the group undergoing laparoscopy had more serious complications: one wound abscess and one failed evacuation versus two cases of urinary retention. In a similar study design, Lundorff et al.[13] compared the rates of persistent trophoblast after these two surgical approaches. The rate was 10 per 100 procedures with laparoscopy versus 7 per 100 with laparotomy (relative risk 1.5; 95% confidence interval 0.4 to 5.8). Again, small sample size severely limited the power of this study. To demonstrate a 50% higher rate of persistent trophoblast in the group treated with laparoscopy (α 0.05, power 80%, and equal allocation of subjects), 2338 subjects would be required.

Numerous descriptive studies[14-33] have been published. These include both case-series reports (Table III) and case reports.[34-36] The patients described in Table III overlap; therefore, the numbers of cases can-

Table III. Laparoscopic management of ectopic pregnancy in selected reports, by type of study

Author	*Location*	*n*	*Complication rate per 100 operations*	*Comment*
Randomized controlled trials				
Vermesh et al.[11]	Los Angeles	60	10	Same rate as laparotomy group
Lundorff et al.[6]	Goteborg	73	42	Lower rate of adhesions than laparotomy group
Cohort studies				
Brumsted et al.[12]	Vermont	50	8	Same rate as laparotomy group
Lundorff et al.[13]	Goteborg	98	10	Higher rate of persistent trophoblast than laparotomy group
Case-series reports				
Soderstrom[14]	Seattle	5	NS	—
Bruhat et al.[15]	France	60	5	Failure to evacuate
DeCherney et al.[16]	New Haven	19	5	Laparotomy because of technical failure
Cartwright et al.[17]	Nashville	27	4	Hemorrhage
Pouly et al.[18]	France	321	12	Failure to evacuate; hemorrhage; pulmonary edema
Bornstein et al.[19]	San Francisco	22	0	—
DeCherney et al.[20]	New Haven	79	4	Hemorrhage; pelvic infection
Debuisson et al.[21]	France	100	3	Laparotomy because of technical failure; deep venous thrombosis
Reich et al.[22]	Pennsylvania	17	0	—
Magos et al.[23]	Oxford	6	0	—
Magos et al.[24]	Oxford	19	5	Laparotomy because of technical failure
Mettler and Semm[25]	Kiel	207	4	Failure to evacuate; hemorrhage
Reich et al.[26]	Pennsylvania	109	4	Failure to evacuate; hemorrhage
Silva[27]	Wisconsin	22	5	Failure to evacuate
Donnez and Nisolle[28]	Brussels	300	6	Failure to evacuate; hemorrhage; persistent trophoblast
Henderson[29]	San Francisco	23	13	Failure to evacuate; hemorrhage
Mecke et al.[30]	Kiel	202	7	Failure to evacuate; hemorrhage; infection
Mecke et al.[31]	Kiel	33	52	Rate of adhesions at follow-up
Letterie and Fasolak[32]	Honolulu	9	0	—
Seifer et al.[33]	New Haven	81	14	Persistent trophoblast

NS, Not significant.

not be aggregated for analysis. Overall, the rates of complications were low, usually 4 to 5 per 100 procedures. The most frequent complications were failure to evacuate the products of conception, hemorrhage requiring a different or repeat operation, and persistent trophoblast requiring repeat operation or methotrexate treatment. The highest reported rate of persistent trophoblast was 14%.[33]

Operative laparoscopy has also been used to treat ectopic pregnancies in uncommon loci. Both Hill et al.[37] and Reich et al.[26] have treated interstitial ectopic pregnancies, and others[38, 39] have managed ovarian pregnancies with the laparoscope.

The quality of evidence to date concerning this indication for operative laparoscopy is class II-2. Neither of the two randomized trials met the requirements for reporting such studies,[40-43] and neither had sufficient power to demonstrate potentially important differences. There is fair evidence (class B) to recommend that operative laparoscopy be used to treat ectopic pregnancies, although the four comparative studies[6, 11-13] suggest that the short-term safety and efficacy are similar or inferior to that of laparotomy. Laparoscopic treatment has not been shown to result in fewer adhesions or higher tubal patency rates than treatment by laparotomy.[44] Long-term follow-up studies and comparisons of laparoscopy versus injection of ectopic pregnancies with methotrexate or prostaglandin are needed.

Ovarian biopsy. Biopsy of the ovary was one of the earliest indications for operative laparoscopy, and a flurry of reports appeared in the early 1970s. One retrospective cohort study[45] described substantial morbidity. It compared the combination of diagnostic lapa-

roscopy with ovarian biopsy, tubal patency studies, and endometrial biopsy in 75 patients versus the same procedures without ovarian biopsy in another 75 patients. The relative risk of being hospitalized longer than 24 hours was 2.9 (95% confidence interval 1.3 to 6.4) for the group having ovarian biopsy. In addition, several serious complications occurred in the group undergoing biopsy, including one transected ureter and one infected hematoma.

Nine case-series reports[46-54] have described ovarian biopsies in 770 patients. Complication rates ranged from 0 to 5 per 100 procedures. Most of these complications were hemorrhage, although one report of 39 patients[51] described a ureterovaginal fistula after an unsuccessful attempt to perform a biopsy of a streak gonad. Two cases of tumor implantation at the laparoscopy site after biopsy of a serous ovarian tumor of low malignant potential have occurred.[55] The quality of evidence concerning laparoscopic ovarian biopsy is class III, and there is fair evidence (class B) to recommend operative laparoscopy for this indication. Nevertheless, the aggregate rate of ureteral injury appears to be about 2 per 1000 procedures, similar to that reported for adnexectomy performed by laparotomy (1 per 1000).[56]

Treatment of polycystic ovary syndrome. Several groups of investigators have traumatized ovaries in the hopes of reproducing the beneficial effect of wedge resection without the attendant risk of postoperative adhesions. One randomized controlled trial[57] compared ovarian electrocautery with either human menopausal gonadotropins or pure follicle stimulating hormone as therapy. The rate of ovulation was equal (71 per 100 procedures) in the groups receiving treatment with cautery or human menopausal gonadotropin. This trial failed to meet many of the reporting requirements for such studies, and it lacked sufficient power to have identified a twofold difference in rates of ovulation between treatments.

One cohort study[58] compared the endocrine response of six women with ovarian cautery versus that of six who were subjected to laparoscopy without ovarian cautery. Androstenedione, testosterone, estradiol, and luteinizing hormone levels decreased after surgery in the group receiving cautery but not in the controls.

Several case-series reports[59-66] ranging in size from 9 to 85 patients have reported ovulation rates of 45% to 92% within 3 months after the operation. The lowest rate[59] was obtained after ovarian resection (multiple biopsies) as opposed to cautery or laser vaporization in the other reports. These reports also corroborate improvements in levels of androgens and gonadotropins after treatment. Long-term effects await further study. No early complications were reported, but a small proportion of patients were later found to have ovarian adhesions.[60,67] Finally, one case-series report[68] described no apparent adverse effects on pregnancies conceived after ovarian electrocautery. The quality of evidence for this indication is II-2, and there is fair evidence (class B) to recommend use of laparoscopy to treat polycystic ovary syndrome that is refractory to therapy with clomiphene citrate.

Adnexal operations unrelated to ectopic pregnancy. One retrospective cohort study[69] compared laparoscopic salpingectomy, salpingostomy, oophorectomy, and ovarian cystectomy in 330 patients with similar operations by laparotomy in 129 patients. Included in the laparoscopy group were 98 women having salpingectomy or oophorectomy for indications other than ectopic pregnancy; the complication rate was 9 per 100 procedures. Comparable morbidity data were not provided for the group having laparotomy. Both selection and ascertainment bias appear likely in the study. Overall operating times were similar for both groups, although those undergoing laparoscopy had significantly shorter hospital stays and recuperation periods.

Several case-series reports[70-74] confirm the feasibility of adnexectomy via laparoscopy. In these reports, which range in size from 8 to 100 patients, the complication rates varied from 0 to 18 per 100 procedures.[70,73] Complications included burns, wound infection, hematoma, hemorrhage, and brachial plexus injury. One report[72] described successful laparoscopic treatment of adnexal torsion in 77% of 35 patients. In addition, a recent case report[75] has documented removal of streak gonads in a patient with gonadal dysgenesis. The quality of evidence for this indication is class III, and there is insufficient evidence to recommend laparoscopy for this indication (class C).

Ovarian cysts. Management of ovarian cysts is one of the most common and controversial applications of operative laparoscopy. Problems with this technique include the inadequacy of cytologic diagnosis of aspirated cyst fluid, recurrence of cysts, and the potential for disseminating or delaying the diagnosis of ovarian cancer. The case-series reports to date,[76-83] ranging in size from 9 to 420 patients, have not resolved these issues.

In an early series of 71 patients, Kleppinger[76] reported a recurrence rate of 33 per 100 procedures when only aspiration was performed, in contrast to zero when fenestration of the cyst capsule was done ($p < 0.01$ by Fisher exact test). In a series of 19 patients Larsen et al.[77] revealed the potential for missing ovarian cancer. They aspirated a cyst from an ovary in a 40-year-old who 7 months later was found to have a serous cystadenocarcinoma in the same ovary. Another patient had a dermoid cyst open accidentally during surgery and required readmission for abdominal pain 1 week later; the complication rate in this series was 11 per 100 procedures. Magos et al.[78] reported a complication rate of 6 per 100 procedures (one laparotomy because

of a technical failure). In a series of nine patients with dermoid cysts, Nezhat et al.[79] reported no complications, although three patients required colpotomy to remove large cysts.

Hasson[80] noted a recurrence rate of 13 per 100 procedures; the likelihood of recurrence of endometriomas varied with surgical technique. With fenestration alone 8 of 9 (89 per 100 procedures) cysts recurred in contrast to 2 of 18 (11 per 100) with fenestration plus coagulation or resection of the cyst lining (relative risk 8.0, 95% confidence interval 2.1 to 30.2).

The largest series to date[81] documented a low complication rate with cystectomy (1 per 100 procedures); complications included two cases of ovarian abscess. These authors found a high sensitivity but low specificity for identifying ovarian cancer with laparoscopy. In a pilot study of operative laparoscopy for cysts in carefully selected postmenopausal women, Parker and Berek[82] noted a complication rate of 12 per 100 procedures, including one injury to the bladder and one to the colon.

The most worrisome report,[83] however, stems from a survey of oncologists concerning laparoscopic excision of ovarian neoplasms that were later discovered to be malignant. Twenty-nine respondents reported 42 such cases. Laparotomy was delayed in 71% of cases, with a mean interval of 5 weeks. Half of these patients had stage II or greater disease. In five cases exploratory laparotomy was never done. One respondent documented a 9-hour attempt to perform cancer staging by laparoscopy.

The quality of evidence concerning management of ovarian cysts is class III, and the evidence is insufficient (class C) to make a recommendation concerning this indication. Operations in which the cyst fluid is drained but the cyst is not submitted for histologic examination can compromise the care of patients with unsuspected ovarian cancer.

Pelvic abscess. Although laparoscopy has long been the standard for diagnosis of salpingitis, few investigators have explored its potential usefulness as treatment. Three small case-series reports[84-86] have described laparoscopy to drain pelvic abscesses and to break up loculations of pus. Ninety-five patients underwent such procedures without reported complications related to the laparoscopy. The quality of evidence concerning this indication is class III, and there is insufficient evidence (class C) to recommend the use of laparoscopy for this purpose. If laparoscopic treatment of pelvic abscesses is subsequently found to have merit, this benefit will have to be balanced against the additional costs and risks related to the operation.

Laparoscopic uterosacral nerve ablation. Prompted by the favorable experience of Doyle[87] with transection of the uterosacral nerves as treatment for refractory dysmenorrhea, two reports have described nerve ablation at laparoscopy. One study[88] of 21 patients without organic pathologic conditions found an improvement in symptoms lasting up to 1 year among women who had ablation by cautery. No benefit was seen among women who had only diagnostic laparoscopy ($p = 0.04$, Fishers two-tailed test). No complications were reported. Although the report claimed to be a double-blind trial, the randomization process was not random, and the allocation was not blind. Hence, this study should be considered a prospective cohort study.

The same author[89] reviewed his experience with 54 such transections; 32 patients (59 per 100 procedures) reported "significant" improvement in symptoms. It is unclear whether subjects in the randomized trial[88] were included in this case-series report. In addition, one case-series report[90] described "laser neurectomy" via laparoscopy in 12 women. The success rate was not specified for this group, although in the total group of 42 patients 71% had little or no dysmenorrhea 1 year after operation. The complication rate was not specified. Nevertheless, at least one ureter has been severed as a complication of coagulation and transection of the uterosacral ligaments in a patient with no pelvic pathology.[91] The quality of evidence for this indication is class II-2, but there is insufficient evidence (class C) to recommend the use of laparoscopy for this purpose.

Ventrosuspension of the uterus. Suspension of the uterus has been suggested as therapy for deep dyspareunia and chronic pelvic pain associated with retroversion. In one case-series report,[92] 72 patients had laparoscopic suspension performed by lifting the round ligaments out of the abdomen and suturing them to the anterior rectus fascia. Only 26% of patients with follow-up at 6 months reported benefit, and the complication rate was 39 per 100 procedures. Complications included 19 cases (30 per 100 procedures) of persistent groin pain and six (9 per 100) wound infections. An earlier case-series report[93] described the outcomes of 100 cases of ventrosuspension. The success rate was high (89 per 100 procedures) and the morbidity rate low (9 per 100). Nevertheless, the median number of days spent in the hospital was 5. The quality of evidence for this indication is class III, and there is insufficient evidence (class C) to make a recommendation regarding this use.

Other applications. Several other uses for operative laparoscopy have been explored. One case-series report[94] has described myomectomy without complications in 17 patients previously treated with gonadotropin-releasing hormone analogs. One case report[95] has documented resection without complications of a noncommunicating rudimentary uterine horn. Two groups[96,97] have published reports of "laparoscopic hysterectomy" in which most of the operation was per-

formed by laparoscopy. In both, however, the procedure was completed by the vaginal route. Because operating times ranged from 90 to 180 minutes, the benefit of this approach as compared with vaginal hysterectomy is not apparent. On the other hand, laparoscopy may be useful in identifying appropriate candidates for vaginal hysterectomy.[98] The quality of the evidence concerning these indications is class III. There is insufficient evidence (class C) to recommend the use of laparoscopy for myomectomy, resection of a noncommunicating uterine horn, and hysterectomy.

Comment

The literature concerning expanded indications for operative laparoscopy consists primarily of descriptive studies. Very few randomized controlled trials or analytic studies have been performed; those that have been done have methodological shortcomings, including low power. Because of these limitations, whether these operations are preferable to laparotomy remains unknown.

A double standard prevails today in gynecologic therapy. Although elaborate clinical trials are required before the use of new pharmaceuticals, comparable scrutiny is not required before new operations are adopted and disseminated. A scientific process determines what medicines our patients may receive; competitive marketing, opinion, and uncontrolled human experimentation determine what operations they may receive. Unnecessary trial and error is particularly troubling in surgery because, unlike a medicine, an operation cannot be withdrawn after it has been administered to a patient.[99]

The recent proliferation of uncontrolled experimentation with operative laparoscopy highlights the need for systematic assessment of new surgical technologies. Many thousands of operative laparoscopies are being performed annually[2] in the absence of solid evidence that they are preferable to other surgical alternatives. As observed by one ethicist, "If a study of the history of medicine reveals anything, it reveals that clinical judgment without the check of scientific controls is a highly fallible compass."[100]

Operations come and go. If they "graze" on the medical commons too long, they may squander valuable time and resources that could have been devoted to worthwhile surgery.[101] Examples of operations performed during this century that were accepted as standards of care yet were ultimately discarded as worthless include colectomy for epilepsy, renal capsule stripping for acute tubular necrosis, surgical correction of ptosis of the internal organs, and operations to remedy constipation.[101, 102] Similarly, hysterotomy and hysterectomy for elective abortion were abandoned in the United States when analytic studies showed them to be inferior to alternative techniques.[103]

Critical appraisal of new operations can help us to avoid repeating these errors. Although some[104, 105] have argued that formal trials are impractical and would impede innovation, these criticisms lack merit. As noted by a distinguished panel of scientists, "There is no scientific or humanistic reason why standards for acceptance should not be equal for all treatments. On the contrary, science and ethics demand our best efforts for equal standards."[106] Clearly, the best effort is the randomized trial.

After the initial feasibility of a new procedure has been demonstrated multicenter, collaborative trials should be started with uniform protocols and outcome measures. Professional societies should initiate and conduct such studies. For example, trials performed under the auspices of the Gynecologic Oncology Group have made important contributions to our management of cancer. Clinical trials, however, can be challenging: it may take years to recruit adequate sample sizes, multiple centers may need to participate, and the costs can be large. If a new operation is shown to be safe and effective, general use could begin with provision for continued surveillance of outcomes and complications as the operation's use grows (analogous to postmarketing surveillance of drugs).[107]

Future research should compare laparoscopy not only with traditional laparotomy but also with other alternatives, such as minilaparotomy or colpotomy. For example, many ectopic pregnancies can be easily managed through a 3 cm abdominal incision with the assistance of a uterine elevator. Alternatively, if the gynecologist must perform a colpotomy to remove an adnexal cyst after dissection with the laparoscope, the possibility of conducting the entire operation through a colpotomy incision warrants evaluation. Many cysts can be deflated and removed vaginally.

The adoption of new technologies often follows a sine-wave curve. Operative laparoscopy is currently on the ascending slope, and enthusiasm may have outpaced critical evaluation. Laparoscopy has unquestionably made important contributions to women's health in the past and will likely continue to do so. Technology assessment, however, is urgently needed to define that future role. If physicians do not provide technology assessment, other interests are likely to do so. In the interim, Gomel's "healthy coefficient of skepticism"[1] must remain high: a 9-hour staging laparoscopy for ovarian cancer is unlikely to be in the best interests of the patient. That an operation can be done through a telescope does not imply that it should be.

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Infertility and endometriosis: Comparison of pregnancy outcomes with laparotomy versus laparoscopic techniques

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OBJECTIVES: The null hypothesis of this retrospective literature analysis was that the superiority of laparoscopy over laparotomy to correct infertility resulting from tubal injury has not been proved because of the lack of well-designed comparative studies. The same was true for the correction of infertility caused by minimal and mild endometriosis.
STUDY DESIGN: A retrospective review of the English-language literature since 1975 was made to ascertain whether laparoscopic surgical correction of infertility caused by tubal injury and endometriosis resulted in an increased pregnancy rate compared with laparotomy techniques. Complication rates associated with laparoscopy versus laparotomy were also compared.
RESULTS: There were almost no adequate studies designed and executed to answer these questions based on criteria established by the United States Preventive Services Task Force. Furthermore, there was no evidence in the reported series that laparoscopic surgical procedures were superior to laparotomy in correcting infertility. The complication rates were similar. There was suggestive, but not yet proven, evidence that laparoscopic surgery with laser techniques may be superior to laparotomy in the management of infertility resulting from moderate and severe endometriosis.
CONCLUSION: Although results and complications were similar, the cost in savings with respect to decreased hospital expenses and loss of work time favor the use of laparoscopy over laparotomy when results are similar and not associated with increased risk. What has not been established are costs and work-time losses for minilaparotomy compared with laparoscopy. (Am J Obstet Gynecol 1992;166: 1072-81.)

Since the first laparoscopic excision of an ectopic pregnancy, the indications and uses for laparoscopies have increased dramatically. New surgical instruments are available, and videoscopic pictures now allow the surgeon's assistants to observe the operative field and more ably assist the surgeon. The advent of laser techniques have added newer and, it is hoped, better and more useful destructive and dissection tools to the surgeon's armamentarium. These truly remarkable surgical tools have found a receptive clinical audience and widespread clinical use. The recently published survey of the membership of the American Association of Gynecologic Laparoscopics[1] contained a summation of responses from 880 physicians representing approximately 24% of the membership. These physicians performed 36,928 laparoscopic procedures during 1988. A conservative projection of procedures peformed by the total membership can be achieved by multiplying by three the procedures done by the 24% of responding members. This yields a value of more than 110,000 laparoscopic procedures performed in 1988. This conservative estimate of gynecologic procedures does not include those performed by physicians who were not members of the Association or by resident physicians in training.

In spite of the widespread acceptance of laparoscopic surgery, the efficacy and safety of such procedures have not been established. Therefore, my assignment was to ascertain the safety and efficacy of laparoscopic surgery in the management of infertility and endometriosis.

It was readily apparent that a clearly defined end point for success or failure could be established for treatment of infertility; the patient either was or was not able to become pregnant after the treatment. For endometriosis success also might be measured by relief of pain and other symptomatic conditions too difficult to assess. For this reason, the success of treatment for endometriosis was also defined in terms of pregnancy outcome. Again, the patient either was or was not able to become pregnant as a result of the treatment.

With clearly defined outcome variables established, laparoscopic surgery for the treatment of infertility and endometriosis could be compared with laparotomy for the correction of these disorders; safety of the two sur-

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6/6/35918

gical approaches could also be compared. Infertility and endometriosis that could be treated by laparotomy and laparoscopic methods alone were then arbitrarily outlined as listed below.

Laparoscopic treatment of infertility and endometriosis

I. Infertility
   A. Tubal injury
      1. Iatrogenic
      2. Inflammation
   B. Ovum retrieval
   C. Unexplained infertility

II. Endometriosis
   A. Endometriosis
   B. Endometriomas

The surgical treatment of tubal injury was divided into reversal of previous tubal sterilization (iatrogenic) and injury caused by an inflammatory process. Reversal of tubal sterilization of laparoscopic technique is not actually an established procedure; therefore, this category could not be compared with laparotomy methods. It is acknowledged, however, that a laparoscopic view of a previous tubal sterilization before a definitive laparotomy may prevent an unnecessary laparotomy if the tube cannot be repaired.

Laparoscopic ovum retrieval for in vitro fertilization and other related techniques has been replaced almost entirely by sonographically directed aspiration and is included only for historic purposes. For unexplained infertility it was impossible to obtain comparative data for laparotomy versus laparoscopy; no specific studies could be found that supported this recommendation in spite of the fact that such a recommendation is made in three standard textbooks of endocrinology. Furthermore, no articles since 1975 could be found in which the authors recommended laparotomy for cases of unexplained infertility. Therefore, data were available only to compare pregnancy outcomes for tubal injury.

Pregnancy outcomes for the surgical treatment of endometriosis have been reported for both laparoscopic and laparotomy methods. The specific category of endometriomas is a hotly controversial area with respect to correction by laparotomy versus laparoscopy. Most treatment outcomes for this indication were reported in terms of successful treatment and rates of recurrence, but comparative studies of specific pregnancy outcomes as a result of laparoscopic versus laparotomy techniques were not identified by this reviewer.

For all the reasons listed above, this review will be devoted to a comparison of the efficacy and safety of laparoscopic versus laparotomy techniques to correct infertility caused by tubal injury and endometriosis.

Table I. Quality of evidence

I	Evidence obtained from at least one properly designed randomized controlled trial.
II-1	Evidence obtained from well-designed controlled trials without randomization.
II-2	Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group.
II-3	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.
III	Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Source: United States Preventive Services Task Force.

Table II. Strength of recommendations

A	There is good evidence to support the recommendation that operative laparoscopy be used for this indication.
B	There is fair evidence to support the recommendation that operative laparoscopy be used for this indication.
C	There is inadequate evidence regarding the use of operative laparoscopy, for this indication, but recommendations may be made on other grounds.
D	There is fair evidence to support the recommendation that operative laparoscopy not be used for this indication.
E	There is good evidence to support the recommendation that operative laparoscopy not be used for this indication.

Source: United States Preventive Services Task Force.

Material and methods

A systematic search of all publications relating to the surgical treatment of infertility resulting from tubal injury and endometriosis was conducted with Medline. Only articles published in English after 1975 were considered. Before 1975 microsurgical procedures were not commonly used.

Final outcome variables for tubal injury were tubal patency, pregnancy, viable pregnancy, ectopic pregnancy, and abortion. Pregnancy alone was chosen as the single outcome variable for treatment of endometriosis. Any complication reported was duly noted. No article was included that did not include information that all other causes of infertility had been excluded. It must be noted that there was *no* standard or uniform use of perioperative antibiotics, steroids, hydrotubation, or assessment of tubal patency. Finally, follow-up intervals varied from article to article and even within

Table III. Tubal injury: Inflammation—salpingoovariolysis, laparoscopy

Author and year of study	USPSTF study type	No. of cases	Outcome					
			Patency		Pregnancy		Viable pregnancy	
			No.	%	No.	%	No.	%
Reich[3] 1987	III (Ovum retrieval impossible)	27	NC		23	85	21	78
Gomel[2] 1983 1989	III (Ovum retrieval impossible)	92	NC		57	62 58*	54 63	59
Fayez[6] 1983	III	24	24	100	12	50	8	33
	TOTALS	143	24/24	100	92/143	64	87/143	61

USPSTF, United States Preventive Services Task Force; *NC*, not cited.

Table IV. Tubal injury: Inflammation—salpingoovariolysis, laparotomy

Author and year of study	USPSTF study type	No. of cases	Outcome					
			Patency		Pregnancy		Viable pregnancy	
			No.	%	No.	%	No.	%
Reich[3] 1987	III	12	10	83	10	83	9	75
Hulka[7] 1982	III	47	NC		15	32	12	25.5
Diamond[9] 1979	III	140	NC		86	61	80	57
Bronson and Wallach[10] 1977	III	35	NC		22	63	20	57
	TOTAL	234	10/12	83	133/234	57	121/234	52

USPSTF, United States Preventive Services Task Force; *NC*, not cited.

groups of patients reported in the same study. Not all reports included life-table analyses of pregnancy outcomes after treatments.

Articles chosen for analysis were separated into these related to tubal injury and endometriosis. Once in these categories, the articles were subdivided into techniques using laparotomy and laparoscopy.

Each article in each category and subcategory was classified according to the rating scale (Table I) reported by the United States Preventive Services Task Force,[2] which was modified from the original methods developed by the Canadian Task Force on the Periodic Health Examination. This rating scale gives greater credence to studies that are subject to less bias, such as randomized controlled studies, and less credence to case reports and expert opinions.

The efficacy and safety of each surgical technique was assessed based on the type of study (Table I), and the existing evidence was considered for each article or group of similar articles. A recommendation was then made on the basis of the United States Preventive Services Task Force scale listed in Table II. If results supporting the safety and effectiveness of laparoscopic procedures were reported in well-designed studies (classes A and B), the technique was recommended for the treatment of infertility resulting from tubal injury or endometriosis. When evidence was inadequate with respect to quality or quantity (class C) no recommendation could be made. If there was either fair or good evidence that laparoscopic surgery was not safe or effective (classes D and E), the procedures were not recommended.

Statistical analysis was performed on a computer program developed by Dr. J. David Erickson of the Birth Defects and Genetic Diseases Branch, Centers for Disease Control, Atlanta, Georgia. Chi-square analysis with Yates' correction was used in all analyses. A difference was considered to be significant if the p value was <0.05.

Results

Infertility

Salpingoovariolysis. Only cases of infertility amenable to surgical correction were considered for review; this

Outcome				
Ectopic pregnancy		Abortion		
No.	%	No.	%	Comment
1	4	3	11	Hulka classification; 23 cases excluded; 14 primary pelvic inflammatory disease; 13 repeat surgery; 6 endometrioma; 2 transient brachial palsy
5	5	5	5	Gomel classification; International Federation of Fertility Societies; classified as severe adhesions in 79 cases and moderate in 13; bilateral in 48 cases; 44 unilateral because the other tube was occluded. *Continuing results published in 1989 article.
		(Spontaneous) 2	2	
		(Induced) 2		
2	8	2	8	—
8/143	5.5	10/143	7	—

Outcome				
Ectopic pregnancy		Abortion		
No.	%	No.	%	Comment
1	8	0	0	Plus 5 salpingotomies.
1	2	2	4	One of the 12 pregnancies was term stillbirth.
1	1	5	3.5	Gross and micro techniques of different time periods were reported; only micro results are included here.
1	3	1	3	Abortions and ectopic pregnancies were estimated from total conceptions, which included several repeat pregnancies.
4/234	2	8/234	3	—

Table V. Salpingoovariolysis outcomes

	Laparoscopy Table III		Laparotomy Table IV		Significance		
	No.	%	No.	%	χ^2 with Yates' correction	Odds ratio	p Value
Patency	24/24	100	10/12	83	—	—	—
Pregnancy	92/143	64	133/234	57	1.77	1.36	0.18
Viable pregnancy	87/143	61	121/234	52	2.63	1.45	0.10
Ectopic pregnancy	8/143	11	4/234	2	3.17	3.40	0.07
Abortion	10/143	7	8/234	3	1.76	2.12	0.18

policy resulted in a merciful narrowing of the possible topics to be considered. When only comparable United States Preventive Services Task Force studies were considered, the choice of papers available for review was further narrowed. For example, listed in Table III are the results of four similar studies[3-6] devoted to the laparoscopic correction of peritubular and periovarian adhesions. It must be noted that in the reports by Reich[3] and Fayez[6] results of their salpingoovariolysis procedures were extracted from more extensive reports of the role of operative laparoscopy in cases of tubal injury. The two reports by Gomel[4, 5] were included because the 1989 review article included an update of the results previously reported in the 1983 report. The data from these four reports were combined because in each study results were reported for treatment of cases of similar severity. In the Reich[3] and Gomel[4, 5] reports the surgery was done because ovum retrieval was judged to have been impossible. In the Fayez[6] report the surgical results were separated into salpingolysis, ovariolysis, and salpingoovariolysis. Only results for salpingoovariolysis were listed in Table III. Reich[3] classified

Table VI. Tubal injury: Inflammation—salpingoostomy, laparoscopy

Author and year of study	USPSTF study type	No. of cases	Outcome					
			Patency		Pregnancy		Viable pregnancy	
			No.	%	No.	%	No.	%
Gomel[11] 1977	III	8	7	87.5	4	50	4	50
Reich[3] 1987	III	7	NC		2	28.5	2	28.5
Mettler et al.[12] 1979	III	38	NC		10	26	10	26
Daniell and Herbert[13] 1984	III	22	17	77	5	23	3	14
Fayez[6] 1983	III	19	6	31.5	2	10	0	0
	TOTAL	94	30/49	61	23/94	24	19/94	20

USPSTF, United States Preventive Services Task Force; *NC*, not cited.

Table VII. Tubal injury: Inflammation—salpingostomy, laparotomy

Author and year of study	USPSTF study type	No. of cases	Outcome					
			Patency		Pregnancy		Viable pregnancy	
			No.	%	No.	%	No.	%
Reich[3] 1987	III	15	9	60	11	73	8	53
Hulka[7] 1982	III	61	NC		12	20	6	10
	TOTAL	76	9/15	60	23/76	30	14/76	18

USPSTF, United States Preventive Services Task Force; *NC*, not cited.

Table VIII. Salpingostomy outcomes

	Laparoscopy Table VI		Laparotomy Table VII		Significance		
	No.	%	No.	%	χ^2 with Yates' correction	Odds ratio	p Value
Patency	30/49	61	9/15	60	0.04	1.05	0.83
Pregnancy	23/94	24	23/75	30	0.45	0.74	0.50
Viable pregnancy	19/56	20	14/75	18	3.35	2.27	0.07
Ectopic pregnancy	3/56	5	8/75	10	0.55	0.48	0.45
Abortion pregnancy	1/56	2	1/76	1	0.25	1.36	0.61

disease severity with the Hulka[7] classification; Gomel[8] used his own classification. Unfortunately, none of the authors used the same operative techniques and perioperative adjuvants.

In spite of the difficulties listed for this category of laparoscopic salpingoovariolysis, the outcome variables were similar enough to compare results among the individual studies ($p < 0.05$) and to compare all these studies with results obtained by laparotomy (Table IV). All of these reports were United States Preventive Services Task Force class III descriptive studies without controls.

There were two transient brachial palsies reported.[3] Unfortunately, it was impossible to ascertain whether these complications occurred in these patients or in other classes of patients also presented in that report.

Listed in Table IV are the results obtained in four descriptive uncontrolled studies (United States Preventive Services Task Force class III)[3, 7, 9, 10] of salpingoovariolysis performed by laparotomy. Once again, dissimilar classification systems were used to classify the severity of disease, and different surgical techniques were used. These studies were chosen for comparison, however, because they were all class III studies with outcome variables similar to those listed in Table III.

There were no differences in significance noted between the individual studies ($p < 0.05$) listed in Table IV. In fact, when the results for salpingoovariolysis by

Outcome				
Ectopic pregnancy		Abortion		
No.	%	No.	%	Comment
0	0	0	0	3 of 9 cases, by laparoscopy; 1 was primary; 5 bilateral.
0	0	0	0	5 primary; 2 previous surgery (1 endometriosis, 1 inflammatory); 10 cases eliminated for valid reasons.
NC		NC		Viable pregnancies not cited but calculated.
1	4.5	1	4.5	6 of 28 patients were excluded for "laparoscopally unrepairable tubes"; CO_2 laser used in this series.
2	10	0	0	Both pregnancies were ectopic.
3/56	5	1/56	2	—

Outcome				
Ectopic pregnancy		Abortion		
No.	%	No.	%	Comment
3	20	0	0	2 of 3 ectopic pregnancies later delivered; the third ectopic pregnancy had a repeat ectopic pregnancy. 5 of 8 viable pregnancies came after second-look laparoscopy.
5	8	1	2	No viable pregnancies as disease worsened as classified by Hulka criteria.
8/76	10	1/76	1	—

laparoscopy were compared with those by laparotomy (Table V), there still were no significant differences noted in pregnancies, viable pregnancies, ectopic pregnancies, and abortions ($p < 0.05$). Ectopic pregnancies did occur with greater frequency after laparoscopic procedures, however. There were no reported complications in the laparotomy series; therefore, it was impossible to compare the two approaches with respect to safety.

The quality of evidence used to compare salpingoovariolysis by laparoscopy versus laparotomy is class III. There is no evidence (class C) to recommend operative laparoscopy over laparotomy for this indication.

Salpingostomy. A search of the literature since 1975 for reports of the efficacy and safety of salpingostomy performed by laparoscopy and laparotomy resulted in the identification of no randomized controlled studies or even cohort studies. Only uncontrolled series of case reports (Tables VI and VII) were identified. There were five series[3, 6, 11-13] reporting similar outcome variables for laparoscopic surgery (Table VI). There were no reported complications, and the differences between these series were not significant ($p < 0.05$).

With respect to salpingostomy performed by laparotomy (Table VII), only two studies[3, 7] were identified in which outcome variables could be compared with results obtained by laparoscopy. The study by Reich[3] was considered closely before it was included because five of the eight viable pregnancies occurred after a second-look laparoscopy. In spite of reports by DeCherney and Mezer[14] that second-look procedures may improve pregnancy rates and decrease ectopic pregnancies, Tulandi et al.[15] refute this for terminal salpingostomies and salpingoovariolysis. Their study was a well-designed, randomized study in which results for 1 year were analyzed. Therefore, the basis of the results of Tulandi et al.,[15] the series by Reich[3] was included in the results summarized in Table VII.

There were better outcomes in the series reported by Reich[3]; however, because of the second-look approach and small numbers, results were not compared between the two series. The results from both studies were averaged, however, and compared with the results obtained by laparoscopy (Table VIII). There were no significant ($p < 0.05$) differences noted between laparoscopy and laparotomy approaches for salpingostomy, and there were no reported complications in any of the studies.

The quality of evidence for salpingostomy either by laparotomy or laparoscopy is Class III. There is insufficient evidence (class C) to recommend one technique over the other.

Endometriosis. The difficulty in comparing results obtained in the surgical management of endometriosis

Table IX. Endometriosis

Author and year of study	USPSTF study type		Pregnancy outcome							
			Mild		Moderate		Severe		Extensive	
			No.	%	No.	%	No.	%	No.	%
Garcia and David[16] 1977	Retrospective cohort with control II-1	Laparotomy macrosurgery	2/3	67	7/19	37	14/49	28.5	—	—
		Control (2 yr)	11/17	65	—		—	—		
			Stage I: Minimal		Stage II: Mild		Stage III: Moderate		Stage IV: Severe	
Fayez et al.[17] 1988	Retrospective cohort without control II-3	Danazol[a]	10/36	28	10/40	25				
							—		—	
		Laparoscopic excisional biopsy plus danazol[b]	21/38	55	22/42	52				
							—		—	
		Laparoscopic excisional biopsy[c]	27/38	71	33/44	75				
							—		—	
Damewood and Rock[18] 1988	Retrospective case description III	Laparoscopic electrocautery	4/12	33	5/13	38	2/8	25	1/6	16

USPSTF, United States Preventive Services Task Force; *NS*, not significant.

Table X. Endometriosis

Author and year of study	USPSTF study type		Pregnancy outcome							
			Mild[a]		Moderate[b]		Severe[c]		Extensive[d]	
			No.	%	No.	%	No.	%	No.	%
Rock et al.,[20] 1981	Retrospective cohort, no control II-3	Laparotomy	28/45	62	48/88	55	33/66	50	6/15	40
Nezhat et al.,[21] 1989	Retrospective cohort, no control II-3	Laparoscopic laser	28/39	72	60/86	70	45/67	67	35/51	69

USPSTF, United States Preventive Services Task Force; *NS*, not significant.

is best illustrated by an inspection of the three studies listed in Table IX. The first, by Garcia and David,[16] was a retrospective cohort study that contained a control group of patients who did not undergo laparotomy for surgical correction of their mild disease. Unfortunately, the classification of severity was based on the 1973 Acosta classification, which is no longer widely used. In addition, there were too few patients with mild disease treated to make this a truly meaningful report. It is included here only because 17 patients with mild disease were *not* treated and 11 (65%) became pregnant. This was, in fact, the only report identified since 1975 in which there was a control series of patients. Schenken and Malinak[19] reported similar success without treatment for minimal and mild endometriosis. Again, it is unfortunate that a more current classification was not used in the study by Garcia and David.[16]

The series by Fayez et al.[17] was matched with that by Damewood and Rock[18] to illustrate their differences and to describe why they cannot be compared. Although both groups of authors used the revised American Fertility Society (1985) Classification for Endometriosis, the varying uses of hormonal therapy made the two series impossible to compare. This, unfortunately, was true for most descriptive unrandomized and uncontrolled series that were reviewed.

The difficulty in comparing results reported in our literature is further substantiated in the Fayez et al. (Table IX) study,[17] in which excisional biopsy alone or in combination with danazol resulted in a better preg-

Classification	*Comment*
Modified Acosta (1973)	Control that had no surgery (mild disease).
Revised American Fertility Society (1985)	a-b = $p < 0.001$ a-c = $p < 0.001$ b-c = NS
Revised American Fertility Society (1985)	"Six months of danazol therapy had been utilized for the six months before conservative surgery or postoperatively for six months in 14 of the patients."

Classification	*Comment*
American Fertility Society (1979)	a + b − c + d = $p \leq 0.05$. Exact surgical technique not described.
American Fertility Society (1979)	a − b − c − d = NS

nancy outcome for treatment of minimal (stage I) and mild (stage II) endometriosis than did danazol alone. Unfortunately, both excellent results were no better than the control results noted in the Garcia and David (1977) study.[16]

I was able to locate two retrospective cohort studies[20, 21] in which the same classification system was used to stage endometriosis and in which both studies reported life-table analyses of successful pregnancies. The results of this comparison are listed in Table X. Both reports also were classified as United States Preventive Services Task Force class II-3 studies. Unfortunately, in the report of Rock et al.[20] the surgical techniques were not described in detail. However, the surgical approach was by laparotomy; therefore, this series was compared to the Nezhat et al.[21] study in which laser and laparoscopy were used.

In spite of the difficulties noted for a comparison of reports of Rock et al.,[20] and Nezhat et al.,[21] they both were judged to be United States Preventive Services Task Force class II-3 studies and both authors used the same classification system (American Fertility Society Classification 1979). There were no reported complications in either series, and there were no significant differences in outcome per class of endometriosis between the two series (Table XI). Thus the quality of evidence is class II-3, but there is insufficient evidence (class C) to recommend the use of laparoscopy over laparotomy. It must be noted in all fairness, however, that Nezhat et al.[21] point out that their success rate was not significantly different from class to class. On the basis of equal treatment results in mild to severe disease, Nezhat et al. claimed that, "Indeed, if results similar to ours are obtained by other reproductive endoscopic surgeons, the obituary of laparotomy as written by DeCherney soon will be published."[21] Based on the meager comparative data available and the United States Preventive Services Task Force classification and recommendation guidelines, this claim appears to be premature.

Comment

A systematic review of the gynecologic literature back to 1975 concerning the efficacy and safety of laparoscopy versus laparotomy for the correction of infertility resulting from tubal injury and endometriosis was conducted. The majority of published papers were primarily a list of expanded case reports (United States Preventive Services Task Force class III) of limited scientific merit. Even worse was the failure of the authors to use the same classification systems for grading the severity of disease they were treating. Worse still was the failure of investigators to use the same, or even similar, perioperative adjuvants, such as antibiotics, systemic or topical steroids, hydrotubations, and various preparations claimed to retard adhesion formation. Standard durations of follow-ups were not used in most studies, and life-table analyses were reported in only a minority of the reports.

My final dispassionate conclusion is that there is insufficient evidence to recommend laparoscopy over laparotomy for the correction of infertility resulting from tubal injury or endometriosis. This conclusion was based on the review of poorly scientifically designed and executed studies, which were all that were available. Also, no conclusions reached were based on economic considerations. Certainly there can be no question that laparoscopic surgery results in a significant reduction in hospital costs compared with laparotomy. Levine[22]

Table XI. Endometriosis outcomes with the American Fertility Society 1979 classification

	Table X				*Significance*		
	Laparotomy (macrosurgery) (Rock 1981)		*Laparoscopy (laser) (Nezhat et al., 1989)*				
	No.	*%*	*No.*	*%*	*χ^2 with Yates' correction*	*Odds ratio*	*p Value*
Mild	28/45	62	28/39	72	0.48	1.54	0.48
Moderate	48/88	55	60/86	70	3.65	1.92	0.055
Severe	33/66	50	45/67	67	3.36	2.04	0.066
Extensive	6/15	40	35/51	69	2.91	3.28	0.087

reported a 49% reduction in overall hospital costs in three categories of patients. His first category included patients undergoing salpingoovariolysis. Equally important is a consideration of recovery time associated with laparoscopy versus laparotomy. Azziz et al. reported convincing evidence that "even the most radical endoscopic procedures required considerably less recuperation time when compared with laparotomies."[23] They also noted, however, that in extensive pelvic procedures, recovery time was prolonged even if the surgery was done through a laparoscope.

What should be done? Certainly, well-designed and well-executed prospective studies *with controls* are essential. The time is long past to continue descriptive studies. The cry that such studies are impractical, unethical, or cannot be accomplished is simply unjustified. In fact, the opposite is true. Today the indications for laparoscopic surgery *almost* approach the technical developments of the equipment. Therefore prospective control randomized studies can be designed. Furthermore, specific outcome variables can be defined, and specific perioperative adjunctive therapy can be specified. Certainly time-life analyses can be made. Modern billing and collections sytems make it a simple matter to compare, prospectively, the costs of newer techniques of laparoscopic surgery with those of conventional laparotomy and with newer techniques, such as minilaparotomy. In fact such studies do not have to be sponsored by the National Institutes of Health or the Federal Drug Administration. These studies can and should be sponsored by the American Gynecological and Obstetrical Society or the American Fertility Society, if for no other reason than that the studies can be completed in appreciably less time and for much less money. For example, in his 1979 book *The Politics of Contraception,* Carl Djerassi estimated that the perfect male contraceptive "would require 12-20 years and at least $15 million before such an agent would be available to the U.S. consumer."[24] Thus our professional societies should sponsor this clinical research and mandate that the newer types of laparoscopic surgery be compared with newer nonlaparoscopic procedures such as minilaparotomy.

In closing, I have not identified objective evidence that "the obituary of laparotomy for pelvic reconstructive surgery has been written," as DeCherney suggested in his thoughtful editorial, "The leader of the band is tired . . .."[25] Certainly there can be no advantage in performing a 8-hour laparoscopic marathon to ablate endometriosis that might just as well have been completed 5 hours earlier through a minilaparotomy incision at the same cost in dollars and postoperative recovery time and with better results and fewer complications. Indeed, maybe the leader of the band is tired for a good reason.

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The need for technology assessment in the reproductive sciences

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OBJECTIVE: The problem confronting medicine is the rapid diffusion of technology without prior broad-based critical peer review. Laser therapy is an archetype of this problem.
STUDY DESIGN: Guidelines are needed to identify those medical and surgical procedures that are experimental and those that are truly "state-of-the-art" technology. To be cogent these guidelines must cover nonbiomedical issues and include comparisons with alternative modes of therapy.
RESULTS: Steps must be taken to augment the number of individuals in our discipline who can provide leadership in technology assessment and health services research. Joint ventures between professional societies and third-party payers are a logical approach to developing and funding technology assessment programs.
CONCLUSIONS: These steps should help ensure that clinical decision analysis and operations research develop as a bona fide scientific discipline within the reproductive sciences. Scientific resources developed in this way should help to improve health care by eliminating ineffective procedures and retaining the technologies that are most effective and economic. (AM J OBSTET GYNECOL 1992;166:1082-90.)

Key words: Laser, technology assessment, clinical decision analysis

Problem

It is not possible in the time allotted to completely cover the subject of laser therapy. An analysis of laser as a therapeutic modality is appropriately the charge of an ongoing task force of diverse experts permanently commissioned for the task. Without continued reassessment, singular assessments of technology may be unfair to important evolving technologies and hazardous to the progress of science. The laser is an interesting example of a technologic frontier in which, because of its intrinsic novelty, the creation of the instrument itself has provided much of the market impetus for development. The majority of analyses of laser therapy in gynecology indicate that laser therapy is no more effective than alternative therapeutic modalities and is clearly more expensive.[1] Laser therapy has a well-established role and precise indications in ophthalmologic surgery and, to a lesser degree, in otorhinolaryngology.[2, 3] In ophthalmology certain procedures cannot be performed without a laser. Someday the surgical laser may assume such a singular position in gynecology, but currently it is struggling to compete on a cost-effective basis with electrosurgical and even radiosurgical techniques.

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More important are the underlying issues spawned by these discussions that transcend any single technology, of which laser, because of its obvious marketability, is a fitting archetype. In recent years technical developments in the biomedical sciences and their growing applications to the diagnosis and treatment of disease have been exponential. The speed of these technologic developments and their rapid dissemination, especially to the public sector, have been largely responsible for the conundrum that we are tactfully trying to address here today: the diffusion of technology without prior broad-based critical peer review.

Increasing competition, demands for financial performance, and the need for a market edge tempt even the most conscientious health practitioners to select highly technologic modes of diagnosis and therapy that are more legitimate in the eyes of the public than in the eyes of scientific peers. The risk inherent in this type of marketplace peer review is that it will become the major determinant of practice patterns and even affect the direction of medical science. The end result will be treatments and procedures that are of uncertain value because they lack the type of solid primary data necessary for scientific validity. Procedurally oriented treatments and their indications are especially constituted to short-circuit peer review and sideslip into our therapeutic armamentarium. Such treatments or procedures receive public acceptance, become medical fashion, and are difficult to countermand. Some type of critical premarket analysis is essential for all technology; it is especially important for laser therapy,

which seems to provide an aura of high technology with emotional appeal to surgeon, patient, and health care administrator that is difficult to resist. Guidelines in this and similar areas of medical innovation are important for the public good and are sure to come from other directions if professional societies fail to decisively lead all phases of technology assessment.

Need

Technology in the broadest sense includes drugs, devices, and procedures. Approval of a medical device such as the laser by the Food and Drug Administration serves only to acknowledge officially that the device can do what the manufacturer claims. The Food and Drug Administration does not regulate the procedures for which a medical device can be used, but does provide approval to manufacturers for a specific procedure for which a device can be marketed. Appropriate use is the function of the health practitioner. The analysis of appropriate use is the domain of health care technology assessment. The primary prerequisite for appropriate-use analysis is the development of guidelines to identify which medical treatments or surgical procedures are clearly experimental as compared with those that are state-of-the-art technology. To develop into such guidelines, a single, uniform instrument for the initial evaluation of new technology, continued analysis of existing practices and procedures is essential. In the past, technology assessment principally emphasized the efficacy and safety of a medical or surgical intervention. This focus derived from the assessors who were physicians, scientists or, rarely, biostatisticians involved in patient care or product development. Technology assessment is now a comprehensive form of evaluation and policy research that evaluates a much broader spectrum of consequences of health care interventions.[4, 5] Analyses of these diverse downstream issues require the skills of individuals trained in such disciplines as economics, sociology, epidemiology, operations research, and psychometry. The shortage of such expertise among us is a deficit that will need to be corrected. For example, the cost-benefit issue is especially relevant to laser therapy, in which large financial outlays and training commitments are necessary. These costs and their benefits need careful quantitation and comparison with alternative therapies by a member of the health care team with special expertise in that area. It must be carefully assessed whether the net surgical outcome for the patient, including quality of life, is different with the laser than with these alternative therapies. The relative efficacy of laser to nonlaser therapeutic modalities and the laser's effect on the healing process in comparable human structures have been studied in only a small number of juxtaposed experiments. The opportunity for an ipsilateral control side and a parallel experiment is rare in human beings. Unilateral blepharoplasty by cold knife and contralateral blepharoplasty by laser do not reveal differences in primary healing.[6] Similar comparisons with similar results have been described for bilateral vocal cord polyps.

In terms of safety the laser might be considered to be more dangerous than scalpel or electrocautery. On the other hand, the laser does facilitate the dissection and ablation of disseminated types of endometriosis by providing enhanced precision, selective tissue interaction, and occasionally improved access to surgical sites via the endoscope. The evaluation of all these distinct aspects of health care interventions, including patient preferences, need for physician education, and psychologic overtones of high technology are now subsumed under the grand rubric of technology assessment or operations research. This new type of evaluation is more complex but potentially more useful than single evaluations of efficacy and safety. If inappropriate treatments could be eliminated through this type of broad critical analysis it would protect patients from ineffective treatments, elevate the standards of clinical decision-making, and perhaps result in the savings of billions of health care dollars each year.

Some of us may be concerned that critical technology assessment will erode our ability to practice as we deem best, create too many regulatory hoops, and limit innovation. Similarly, medical researchers, pharmaceutical manufacturers, and producers of medical devices fear that technology assessment will inhibit the development and diffusion of new forms of technology. These fears may be justified, but even without our leadership the new technology assessment is here to stay and will probably have a large impact on medical practice in this decade. A large number of diverse organizations have active programs in technology assessment and cost-effectiveness analysis. Along with the American Medical Association these include the National Blue Cross/Blue Shield Association, Emergency Care Research Institute, American College of Physicians (Clinical Efficacy Assessment Program), United States Public Health Service's Agency for Health Care Policy and Research, and Health Insurance Association of America. Recently Rockwell International Corporation, concerned with employee health costs, developed a large in-house staff to assess health care practices and limit health insurance coverage.[7] Similar cost-containment efforts by third-party payers with only select professional input may have a ricochet effect, resulting in the abandonment of effective technologies and the retention of ineffective ones unless an adequate and dynamic assessment process involving all members of the health care delivery team is developed.

Recommendations

There is a need for operations research and technology assessment in which obstetricians and gynecol-

ogists play an active and, preferably, a leadership role. Within the framework of our specialty we need to stimulate a public dialogue that will help us develop a systematic and uniform approach to technology assessment. The American College of Obstetricians and Gynecologists has already begun to provide valuable input to the Agency for Health Care Policy and Research. To improve our ability to set priorities and evaluate the technologies in our discipline, certain issues must be addressed: intellectual resources, location, funding, and research.

Intellectual resources. Obstetricians and gynecologists who have training in quantitative methods are in short supply. There are few individuals in our specialty who possess the necessary consummate skills, critical faculties, and specific interest in technology assessment. A few departments of obstetrics and gynecology have wisely recruited and retained singular faculty with these talents. To date it has been perceived as an important problem but has not received educational or training emphasis. The need to develop *intellectual resources* within our discipline who can provide leadership in this area is self-evident, but the strategies are less apparent. It is not easy because it requires the development of physicians who have skills outside the ordinary idiom of obstetrics and gynecology. These cross-skills should extend to sociology, economics, epidemiology, public health, psychology, statistics, and other disciplines. Unique opportunities exist for physician scholars who have expertise in these multidisciplinary skills related to health services research and technology assessment. The development of a handful of such dexterous individuals would probably cover our leadership needs for the next decade.

Currently there are a limited number of training grants available from the Agency for Health Care Policy and Research, National Institute of Mental Health, National Institute of Aging, and Departments of Defense and Veterans Affairs. The 1990s will surely see a more expansionary posture with respect to health services research and training. The availability of training grants and the development of role models in technology assessment are essential to the development of such intellectual resources in our discipline. Perhaps a competitive award to fund individuals for careers as physician-scientists could be used for this purpose. Through their emphasis on prevention and screening, established pediatricians have already begun to galvanize new pediatricians in the direction of health care planning and assessment careers. Similar role models are needed from within our discipline. Individuals with talent along these lines should be encouraged to pursue graduate degrees in public health rather than subspecialization. Residents and fellows in our training programs should receive a broader education in all aspects of clinical decision-making, with a greater emphasis on epidemiology and biostatistics. The inability of our trainees to weigh competing claims for different forms of therapeutic interventions is a major contributing factor to the hypertrophy of technology and the atrophy of critical judgment in our discipline.

Location of activity. Government, third-party payers, insurers, and professional societies are not the sole repositories of health care technology assessment. Assessment activities and research also take place in medical schools, schools of public health, policy centers, and departments of economics, business, sociology, and political science. A directory compiled by the Association for Health Services Research (1982) lists 37 such centers that are based in or are affiliated with academic institutions.[8] Their primary, although not necessarily exclusive, mission is the conduct of health services and policy research. It would seem both appropriate and practical that the location for an interdisciplinary task such as technology assessment should be from within the professional society whose members are primarily engaged in the use of the technology. Professional societies are in a unique position to learn from experience, a critical factor in technology assessment. Societies are also better able to suggest modifications in technology and develop an orderly system of priority evaluation. In this way operations research and clinical decision analysis could develop as a bona fide scientific discipline within the reproductive sciences. It would elevate clinical decision-making from an art to a science, help to attenuate observer bias, and be no less a scholarly pursuit than other forms of basic science investigation. Within a professional society all this might come under the broad umbrella of health services assessment and could include liability issues related to the use of these technologies. A health services research and appraisal program within a professional society would also foster the development of a plan to identify the type of data to be collected and a system for performing this task. A clearinghouse to gather this type of information and make it readily accessible to health care providers and researchers would be invaluable. Society-based activities would allow the optimal performance of many of these activities.

Funding. Undertaking an assessment of technology and other utilization-management issues is particularly expensive. In Canada industry has formed a partnership with government in assessing technology through the Medical Research Council/Industry joint program to fund technology-based research. Perhaps industry and third-party payers would be willing to enter an agreement with a single professional society to fund technology assessment and health services research in obstetrics and gynecology. Industry might be willing to work with the society to control costs and to develop a

better understanding of the scope, limitations, and indications for the technology under analysis. A system that capitalizes on the strengths and resources of a free-market economy and a private-public organization would be most appropriate and less susceptible to political meddling.

The American College of Physicians has been involved with health care technology assessment since 1981 through its Clinical Efficacy Assessment Program. The program initially was funded by a 3-year grant from the Hartford Foundation, but is now fully funded by the American College of Physicians. This project evaluates medical tests, procedures, and therapeutic interventions within the purview of internal medicine and its certified subspecialties. Blue Cross/Blue Shield supported an assessment of diagnostic testing that was performed jointly with the College. Reimbursement for outmoded and useless procedures was stopped and resulted in a significant reduction in health care costs. Blue Cross/Blue Shield is now spending approximately $60 million to fund a randomized trial to evaluate autologous bone marrow transplantation in patients with breast cancer. A joint program has been developed by the American College of Cardiology and the American Heart Association to asssess the role of invasive versus noninvasive procedures in the diagnosis and management of cardiovascular disease.[9] Currently the Health Insurers of America are supporting a large study in collaboration with the American Urological Society to assess alternative therapies for benign prostatic hypertrophy. The current challenge for obstetrics and gynecology is how to join forces with other organizations for funding and effective decision-making. If professional societies hesitate to be involved, the most likely scenario in the United States is that all assessment activities will be coordinated by the Agency for Health Care Policy and Research, with professional societies functioning as resources in a consultative capacity.

Research. Useful methods do exist for assessing medical technologies, but they need improvement. A major issue in health services research is whether experimental control is a prerequisite for the establishment of causal relationships. Methods other than randomized controlled trials need to be investigated to define their role in technology assessment.[10] Research is especially needed on methods of economic and psychometric evaluation. A high technology procedure is frequently selected for a common diagnostic problem like headache because patient and physician alike cannot deal with the psychologic ramifications of uncertainty. Methods are urgently needed to quantitate the role of psychologic factors in clinical decision-making.

Beyond the reasearch problems of special methods there is the ancient problem of assembling information from a variety of sources and integrating the results. Biology and numbers need to be integrated in plausible and creative ways. Data pooling as a mechanism to improve the quality and timeliness of evaluations of diagnostic technology needs to be explored in obstetrics and gynecology. Greater effort and resources are needed to obtain solid primary data about medical procedures in use. As with laser therapy, it can be seen repeatedly that the obstacle to evaluation is the lack of randomized, controlled studies that are necessary for cogent assessment. Lack of information is a uniformly significant problem. A final major challenge within our own profession is to expunge those stereotypically attitudes that insist that clinical decision-making is beyond critical analysis.

Currently there is a variety of funding opportunities available on a competitive basis through the Agency for Health Care Policy and Research for the performance of technology assessment. This 2-year-old agency received $90 million in appropriations for technology research during 1991. This represents an increase of $41,567,000 over fiscal year 1990. Information concerning this funding source needs to be more widely promulgated and even rhapsodized within our discipline. This funding is largely available through the Medical Treatment Effectiveness Program, which has received an appropriation of $68,500,000 for fiscal year 1991. This area is primarily designed to evaluate the effects of alternate treatments on the outcome realized by the patient. Research in this area is receiving increasing emphasis because of concerns for health care costs and quality. A greater proportion of monies flowing to departments of obstetrics and gynecology for technology assessment would increase the general awareness of these important issues within our discipline.

The development and pervasive diffusion of technology for minimally invasive procedures, in part catalyzed by laser therapy, will compel us to address all of these issues over and over again in the next decade. The emerging technology and the increasing similarity of instrumentation between the operative endoscopist, catherologist, and interventional radiologist should be a stimulus for cross-fertilization.[11] Cross-talking and formalized interaction between all of these disciplines would help to avoid duplication and is essential for a more uniform evaluation of new and existing technologies.[12] Our assessment capability should also be indirectly enhanced by new legislation (Medical Devices Act of 1990, Pub L No. 101-629) effective November 28, 1991, mandating a review and reporting program for any malfunction of a medical device. This program covers all regulated medical devices approved through the Food and Drug Administration's Center for Medical Devices and Radiobiological Health. This mandatory reporting requirement transfers the burden of

monitoring and reporting medical device malfunction from the manufacturer to the institutions where the medical devices, such as lasers, are being used. Sharing this information across disciplines should avert the repetition of technology-related error and more accurately portray the frequency of laser incidents.

Surgery of the future

Because of its promise, laser technology has at times been clouded by hyperbole, illusion, and myths that have grown like Jack's beanstalk. The realistic future of laser surgery rests on a fuller appreciation of the basic physical and chemical mechanisms by which light interacts with organs and organelles. These insights will establish when it is right to use the laser and when it is not. Recognizing when a scalpel or a $5000 electrocautery knife can be more appropriate and less risky than a $100,000 laser device is pivotal to the continued success of laser technology in medicine.

Apart from laser biochemistry, a second reason that will ensure the survival of endoscopy surgery is the development of technology that will enable computers to generate and manipulate three-dimensional video images. This is not far in the future. A computer monitor will generate a bright, three-dimensional image of the patient's pelvis from data it receives from a magnetic imaging device. Using the three-dimensional image to reconstruct the original image on a television monitor can help us to appreciate the shape of normal anatomic structures as well as the dimensions of disease. Although these techniques require a complex combination of optical, mechanical, laser, and computer technology, the objectives are obtainable. These live computer-manipulatable, three-dimensional images should be available within 5 to 10 years. Surgeons unable to function with current two-dimensional imaging will be back to the more comfortable spatial dimensions of the real world. At the same time advances are continuing in laser technology on many other fronts. The excimer laser uses instantaneous bursts of light and does not leave a burnt, charcoal-like surface.[13] There are many new techniques for trapping and manipulating atoms, dielectronic particles, and even organisms with light forces.[14]

Only hermetically controlled trials carefully carried out over the next few years will clearly codify and define the true potential of the laser. In the meantime it is incumbent on all of us to assess technical "advancements" in a methodic, scientific, and ethical manner. It is important to keep a balance between clinical needs, technical capabilities, and costs. More careful evaluation of clinical efficacy is especially needed as additional high-cost diagnostic measures and therapeutic interventions are proposed.

In the last analysis, whether surgery occurs at the level of the patient, organ, or cell, lasers offer physicians and researchers an unprecedented and precise set of optical tools. Only time and good clinical studies will tell whether these tools are used most effectively and appropriately for the patient's well-being.

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Editors' note: The three manuscripts presented at this panel were revised after these discussions were presented.

Discussion

Dr. Alan H. DeCherney, New Haven, Connecticut (Fellow). At the Twenty-seventh Annual Meeting of the American Gynecological and Obstetrical Society in the year 2017, three distinguished members were out playing a sedentary game of golf at the Homestead: Drs. McDonenuff, Rymes, and Cant. As luck would have it, lightning struck and all three perished immediately. Hustled off in sleek white limousines, they were cleaned up, dressed in white caftans, and informed that tomorrow would be Judgment Day. All three slept poorly. McDonenuff ruminated over every precise issue of his life, thinking as to what would be the significant event. Rymes tossed and turned. His head filled with an end-

less stream of numbers and formulas, none of them making any sense. Yet he knew that somewhere in this conglomeration would be his comeuppance. Cant probably slept the best, although frequently there was tumultuous yelling of meaningless aphorisms—things that he had said during his lifetime, none of which he thought he would ever be accountable for because he did not understand then when he said them.

At last, dawn arrived. They were ushered in single file before the judge, Ken Righteous, who stated that the custom here was to look back on the date that you died and throughout the years, find the one day that was significant, and judge from your actions on that day whether time immemorial would be spent in heaven or purgatory. Ironically, the day chosen for this group was the same: September 7, 1991. Judge Righteous said, "You have probably forgotten that day, but that was the day you debated laparoscopic surgery and laser technology. The question here is an easy one, and it is the age-old question of progress versus status quo, liberal versus conservative philosophy." And he stated that judgment in their individual cases would be made based on retrospective wisdom. If their ideas enhanced progress, that would be looked on favorably; however, if their comments on that day were meant to impede progress, that would be weighed against them.

He warned each of them that their arguments must be philosophically sound, because in life that is what counts.

He then turned his attention to Rymes. "Rymes," he said, "you probably remember that in my former life I was a well-known moral philosopher and, just to refresh your memory, a moral philosopher is one who does the right thing based purely on logical evidence. I was quite famous for the quip, 'It is what your grandmother would do.' You have brilliantly described the litany of techniques, dissected this subject thoroughly, and implicated a 'quality of evidence chart,' but, alas, your conclusion was; 'We should not move ahead unless randomized control studies are carried out.' This is an antique argument, and one must ask, 'Why have the dexterous brotherhood never been able to carry out this type of study?' You describe the Vermonter's study that says to demonstrate a 50% higher rate of persistent trophoblast in ectopic pregnancies treated by laparoscopy 2338 subjects would be required. Sarcastically I say, is this practical? Are there not too many variables in surgical studies to really do random controlled studies? Is it not contrived to think that we can study, as you suggested, these surgical procedures with the same scrutiny that we study medication? After all, surgeons are not cognitive procurers of soups and potions.

"Why aren't descriptive studies good enough for complications? Although you've tried to obfuscate the picture with a series of Cartesian arguments, I need not remind you that Descartes, in the search for truth in which all philosophers are employed, believed that a numeric formula providing real truth was unattainable. I think that, although your suggestion for randomized control studies is comprehensive, it is impractical and perhaps illogical. Yet in your defense, Rymes, I offer the following rhyme by the former Poet Laureate of the United States, Howard Nemerov:

> Praise without end the go-ahead zeal
> of whoever it was invented the wheel;
> But never a word for the poor soul's sake
> that thought ahead and invented the brake.

Next, McDonenuff was ushered into the dock. Over the quivering, emaciated Dr. McDonenuff, Judge Righteous stood to his full height, his disheveled red hair like a sunburst and roared "Technology assessment!" Then he sat down.

"McDonenuff," he said, "we have just heard Rymes's procedure assessment paradigm. Your argument is the contrary: a technology assessment paradigm. Once again a logical assumption, but extremely flawed, I am afraid. By your own admission you discuss the improvements made in ophthalmology and otolaryngology with the use of new technology, the laser, to be precise. On that day in 1991 you discussed the failure to prove a 'cost-effective basis' of laser technology when comparing it with an electrosurgical approach. These were good arguments, and I was really excited. You talked about the system being driven by the competitive and business nature of medicine; this was timely, for it was in the late 20th century that medicine became a profession once again after a brief sojourn as a job.

"My heart went out to you in empathy when you discussed the faults with a public-driven rather than a profession-driven system. But, alas, your solution left me disappointed. Regulatory bodies? Just the cost of commissions to study technology assessment would be prohibitive. What would be the chance that industry would be motivated to be a partner in technology assessment?

"You boldly suggested, and also recognized the impracticality and the cost of, the sacred randomized controlled study. You have used Mills's utilitarian argument. Although the premises were logical, the solution was utilitarian but illogical.

"But I was most surprised, McDonenuff, when I recall that fateful day 17 years ago when I discovered that you, yourself, were addicted to technology. Suggesting an empiric approach to animal studies, why not the same for patients? And then you titillated us with the use of three-dimensional images for carrying out surgery.

"Progress mediated by commissions, bureaucrats, and entrepreneurs—a paradox!"

Righteous looked somewhat euphoric as Cant strutted toward the defendant's dock. A man who made the analogy of a laparoscope being just another sword in the belly would provide clear insights into this conundrum. Maybe he would be the one to point out that the day of "show and tell" is over and that the time for honest intradisciplinary professional assessment of the complications and effectiveness of these techniques was at hand. Perhaps Cant, with his more focused mind, could cut through the mass of data provided and suggest even quasi-conclusions and guidelines. But what

we saw that day was a marriage of Humian cynicism and Texas homily. "Nothing works, there are no dramatic results, and we are only cutting hospital stay by 1 day." Righteous said, "At best, using quality of evidence criteria, you are able to concede that some of the studies reached the nirvana of II-3 in the *Book Grimes*. But basically you were inundated with data."

Banging his gavel on the table, Judge Righteous said, "Enough. I have come to profound conclusions and I am ready to pronounce eternal judgment on these three. I have devised a special purgatory for two of them. One is condemned to listen endlessly to the George and Ira Gershwin song "They All Laughed":

> They all laughed at Christopher Columbus when he said the earth was round.
> They all laughed when Edison recorded sound.

The other is condemned to listen endlessly to the Cole Porter tune "I Get A Kick Out Of You":

> My story is much too sad to be told,
> but practically everything leaves me totally cold.
> I get no kick from champagne.
> Pure alcohol doesn't thrill me at all.

And the third, because his arguments were most logical, used the philosophic precept correctly and, most important furthered progress in this important field rather than impeding it by being a naysayer, will go to heaven and for eternity will treat endometriosis in infertile patients with a laparoscope and a laser."

Judge Righteous then exited the chamber humming the final bar of that Gershwin tune, "Ha, Ha, Ha, who's got the last laugh now!"

DR. ROY M. PITKIN, Los Angeles, California. I would like the panel, particularly David and Norman, to give an opinion as to what extent, if any, the indication for an operation is influenced by its cost, cost being defined in the broadest possible sense to include financial costs and risks and inconvenience.

Specifically, a couple of the operations you mentioned, lysis of adhesions and section of the uterosacral ligaments, were not done by laparotomy for the last 40 years but are now being revised and done by laparotomy. If they were not indicated by laparotomy, does the proposition that they can be done with less cost, defined in that broad way, make them a proper indication for laparoscopy?

DR. GANT. Roy, I am glad you asked the question. There is absolutely no doubt that if the endometriosis is mild or minimal disease, stage I or II, you get just as good, if not better, pregnancy results by doing nothing.

DR. MCDONOUGH. Your point, Dr. Pitkin, is an interesting one. Previously discredited macrosurgical procedures (presacral neurectomy, ovarian wedging, etc.) are born again when performed through the endoscope. Apart from some unsubstantiated claims that adhesions are less, etc., there is no logical reason to assume that they have undergone any type of scientific metamorphosis. I take issue with Dr. Grimes on the efficacy of ovarian drilling. There are approximately 12 or 13 publications on the use of this procedure in polycystic ovary syndrome. The outcome looked at in the majority of these publications is the postoperative restoration of steroid levels to normal; comparisons are made with steroid levels in a group of normal ovulating controls. An appropriately matched control group of anovulatory subjects was not used in any of these studies, and ovulation restoration in the treatment group was a therapeutic end point in only a few. As Dr. Pitkin indicated, when analyzed appropriately, a discredited macrooperation (ovarian wedge resection) is no more effective than a similar procedure done endoscopically. We are reinventing the wheel for all of these operation fossils.

DR. GANT. May I add just one other thing? The classic example is that the final step in a negative infertility evaluation must be a laparoscopy. I could find no one who advocated laparotomy for this, and yet I found no one who could report on the incidence of something they found that they could treat that resulted in pregnancy. This number was quoted as 50% in one of the textbooks, but there was no reference.

DR. GRIMES. Let me respond to Paul's point. There is one randomized clinical trial I am aware of from Kuwait that did have three arms. One was ovarian drilling. The other two were medical therapies, and they found comparable rates of ovulation in that one study.

SECRETARY SCOTT. Dr. Pitkin, you mentioned that operations previously discredited and abandoned are now being advocated and performed laparoscopically. What role should be expected of journals, editors, and academic departments in preventing this sort of situation from developing?

DR. PITKIN. I will ask our resident philosopher DeCherney whether that is the classic example of a rhetorical question or begging the question. It is one or the other, but the implications of what you say I would agree with. The peer-review process has to decide, and that means that journals, in particular *reviewers* of journals, have the responsibility to make certain that conclusions are based on the data presented.

Varying studies or varying analyses of published works show that somewhere between 30% and 50% of the time the conclusions stated, usually in the last sentence of the abstract of the paper, are not justified when examined by independent review; this would be an example of that.

DR. MCDONOUGH. One thing I have been impressed with is the ability of metaanalysis to point out the strengths and weaknesses of the literature in the areas we are discussing today. These weaknesses should stimulate a certain amount of soul-searching among all of us concerning standards for scientific publications. Definitive answers to most of the questions we are discussing will not be available until the appropriately designed studies are performed.

DR. CARL LEVINSON, San Francisco, California. Just one comment for each of the speakers. It has been interesting to me to listen to the Russians, all of whom speak English. I think Dr. Grimes unfortunately missed a good many reports dating back to 1973 from the group in Clermont-Ferrand headed by Dr. Bruhat;

many of these studies are truly comparative studies. I recommend that we go back and look at some of those. They are ongoing and they are very, very good.

As far as evaluating laparoscopic surgery for endometriosis, Norman, could we consider those patients who have symptoms and relief of symptoms by surgery and dismissal the same day? It really is quite productive. And Paul, one of the things Alan mentioned was the assessment of surgical skill. When you talk about a technical procedure, technology, and the use of that technology by human beings, how do we assess the use of the technology?

I have been to many centers, and I can tell you there are some people who know how to use the laser and there are many people who do not.

DR. GRIMES: I will respond first. Regarding the publication by Dr. Bruhat, there are a number of his articles in my reference list. Those that were published in French are excluded, as I explained.

DR. GANT. My literature search with Medline was in English and went back to 1975 and because I could not compare so-called modern microsurgery with the macrosurgical techniques before that time.

With respect to the symptoms within endometriosis, I accept the criticism that you are making, but I excluded symptoms and qualified that in my presentation and in the paper because it is most difficult to compare study with study with study. I picked clean end points that I could measure—either the women were pregnant or they were not—as a scientific analysis of these techniques, and I limited them only to whether the pregnancy was successful or not.

I acknowledge that many patients are made symptomatically better. I am also reminded that in drug studies many patients who get the placebo are made symptomatically better as well. And a laparoscopy procedure is a very expensive and dangerous placebo.

DR. MCDONOUGH. This quantitative process (meta-analysis) that we are discussing does not provide an evaluation of surgical technique, but the appropriateness of the procedure, treatment, or intervention. It may be a small consolation to patients to know that their operations, though inappropriate or unnecessary, were performed with great skill. In addition to pooling the quantitative results of many studies, I spoke to quite a few people with the Agency for Health Care Policy and Research, third-party payers, and individuals from private foundations who do technology assessment. Much of the technology assessment done by private foundations is generic in nature, meaning that comparisons with alternative forms of therapies or interventions are not performed. On the other hand, third-party payers and the Agency feel strongly that cogent assessments must include comparisons with other alternative forms of therapy and with no therapy at all.

DR. GANT. Carl, you were emphasizing that the surgeon must be accomplished. Let me assure you I read no paper in which the author did not know how to do what he or she was describing.

DR. KAMRAN S. MOGHISSI, Detroit, Michigan. As usual, the truth appears to be somewhere in between. I think these procedures have merit, but there are also abuses. I have a very difficult time conceiving that we will be doing hysterectomy and other operations that were not mentioned by the panel such as myomectomies through the laparoscope. A myomectomy that is done through a laparoscope probably need not be done.

Often I have seen videos of these so-called myomectomies leaving an enormous crater in the uterus, and these craters are left to heal by themselves. I shudder to think what happens in the subsequent pregnancy. I think there were about a dozen papers in the early 1970s when diagnostic laparoscopy came into use indicating that in patients with unexplained infertility who were subjected to laparoscopy somewhere between 30% and 50% had additional pathologic conditions that were not detected by usual diagnostic techniques. I think those data are the basis of the mention in a number of textbooks that a laparoscopy is mandatory in patients with unexplained infertility.

DR. GANT. In those articles they were limited to mild or minimal endometriosis where the operation either by laparoscopy or by laparotomy achieved the same results as no therapy.

DR. MOGHISSI. Many of these patients have endometriosis, but others have had other lesions such as peritubal adhesions that are not detected by any means except laparoscopy. I think we have to put these in the proper perspective.

One additional comment in support of what I think Paul mentioned: I have looked at the review of the literature comparing laparoscopy with electrocautery. I cannot find evidence that there is difference between cautery and laser with respect to suppression of endometriosis or improvement of fertility.

Now it is quite apparent that it costs about $70,000 to $80,000 to install a laser in any operating room. Cautering costs a few thousand dollars. I think that is another area that needs to be very critically reviewed.

DR. MCDONOUGH. The reason I stressed ophthalmology is that there are certain operations in ophthalmology that cannot be performed without a laser. If you want to traverse normal tissue and affect an area in the retina, you must use a laser. In gynecology laser therapy is easily interchangeable with electrosurgical and even radiosurgical techniques.

DR. GRIMES. I would like to respond to Dr. Moghissi as well. He is correct. There is at least one report in the *Journal of Gynecologic Surgery* describing laparoscopic myomectomy after treatment with gonadotropin-releasing hormone analogues to make the fibroids shrink.

With regard to Dr. DeCherney's earlier comments about sample size, I think that leads naturally into praise for groups such as the Gynecologic Oncology Group, who have mounted randomized clinical trials nationwide that have contributed so much to our information. I think this is clearly needed in the area of laparoscopy as well.

I do not want to be on record as opposing progress

in laparoscopy. What I do oppose, however, is fadcism in surgery. We all recall 15 years ago the epidemic of transsphenoidal hypophysectomy we created in this country that promptly went away once we took a critical look at the data.

Dr. Thomas Kirschbaum, Bronx, New York. You and I feel very much the same with regard to evolution of knowledge.

On the other hand, at the heart of some of Alan's comments is the natural history of the new idea. New ideas enter our field as a result of human ingenuity and the desire to academically advance and to do a better job; soon monetary influences raise their heads. The flood of enthusiasm for new procedures undergoes the bell-shaped curve with respect to time.

Those are strong forces. They are not bad forces, and it seems to me that they gradually play their way out. People make enough money, get tenure, become professors, and establish a position in the world; then their instinctive self-motivated ambition starts to fade.

People come along and make rational judgments 20 to 30 years after the inception of the idea unopposed by enthusiasts and entrepreneurs. The potion satisfies the entrepreneurs, it satisfies those who need academic advancement and in a way it satisfies our gripe about the lack of critical appraisal of new ideas.

Out of that process comes progress, I guess. I think that is Alan's point. Messy as it is, irrational as it is, that is the way we behave around the introduction of new ideas of possible merit to our field.

Dr. McDonough. Tom, you are relying on "natural" selection pressures operating over time to eliminate ineffective, harmless, and harmful treatments. Selection pressures may work over eons in biology so that only the best survive, but one of the principal problems in medicine is that many useless, ineffective, and occasionally harmful procedures are retained. As a patient I would not want to be innocently caught in the slow, gradual evolution of medical innovation.

Dr. Kirschbaum. No. Not all results are good.

Dr. Gant. One example of the result not being good is the Usher regime popularized to treat hyaline membrane disease by administering sodium bicarbonate. To make a baby or any fetus bleed into its head now, all you have to do is make it hyperosmolar. This was done in humans for a long period of time on the basis of an original study that "almost reached statistical significance," $p \leq 0.06$.

Dr. John L. Lewis, Jr., New York, New York. I have a brief comment that is both cynical and optimistic. I think we are going to get the studies that you want to prove efficacy, but they will not necessarily come as quickly from within our group as from outside. Third-party payers now fairly routinely refuse to pay for phase 1 and some phase 2 chemotherapy trials on the basis that they have no proven efficacy. The first time they say they will not pay for surgical procedures before the development of evidence of efficacy, we will begin to get the studies very quickly.

Dr. John Larsen. I have listened with some interest to the projections of regulation and the econometrics; however, I have not heard anyone talk about teaching. When the group puts forward its recommendations, teaching is a point over which this society of scholars, chairmen, and professors might be able to exert some control more readily than they could over expensive, ideally controlled studies or regulation of industry. As you go forward with this, would you consider describing what new techniques the prudent academic department should teach its trainees so they may go out in the community and earn a living?

Dr. Grimes. I think that is a very important point. One of our jobs as academicians is to help define what is worth teaching.

Dr. McDonough: I think we need to put more emphasis on clinical decision analysis and spend more time teaching quantitative techniques to our residents and fellows. This or any other professional society cannot do technologic assessment because it is too expensive. You have to figure out some way to join forces with industry or third-party payers who can support this type of activity. Other societies are adopting that strategy. The American College of Physicians, the American Urological Society, and the American Pediatric Society are becoming copartners with third-party payers for technology assessment. They are starting with well-focused projects that will expand to other broader projects because insurers are motivated to critically evaluate alternative forms of therapy, including the biomedical aspects, for economic reasons. If we do not get into the other nonbiomedical issues we are going to be standing on the sidelines. We cannot remain comatose on this topic; otherwise, technology assessment and this type of operations research will be done exclusively by third-party payers. From their perspective, assessment must be comparative to be relevant and address a broad range of issues, including efficacy, safety, cost, patient preference, liability, and psychologic issues. It is clinical decision-making elevated or reduced, depending on your perspective, to a multidisciplinary science. The fine art of clinical decision-making is in the process of being demystified by the health care analysts.

Dr. Gant. The surgeons are doing this with cholecystectomy, for God's sake. Why can we not do it? We taught them how to use the swords.

Dr. McDonough. But you need money to do technology assessment, and money is in short supply.

The effect of cesarean section on intraventricular hemorrhage in the preterm infant

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OBJECTIVE: The null hypothesis is that active labor is a more important factor with regard to both timing and progression of periventricular-intraventricular hemorrhage than is route of delivery. Infants delivered by cesarean section after entering the active phase of labor will behave in a manner similar to that of previously studied infants delivered vaginally as to when periventricular-intraventricular hemorrhage occurs and frequency of progression.

STUDY DESIGN: The 106 infants of 85 women delivered by cesarean section were the subjects of this study. Forty-six infants were in the no-labor group, 33 in the latent-phase labor group, and 27 in the active-phase labor group. Head ultrasonographic examinations were performed at delivery, at 1, 6, 12, and 24 hours, and then daily for the first 7 days of life. Continuous variables were compared by one-way analysis of variance among those infants with no hemorrhage or with periventricular-intraventricular hemorrhage. Categoric variables were compared by χ^2 analysis and Fisher's exact test when appropriate. A p value of <0.05 was considered significant.

RESULTS: There was no difference in the frequency of early hemorrhage (≤1 hour of age), late hemorrhage (>1 hour of age), or overall periventricular-intraventricular hemorrhage in the infants not in labor, in latent-phase labor, or in active-phase labor at the time of cesarean section. However, the frequency of grade 3 or 4 hemorrhage and the progression of hemorrhage were significantly higher in the infants whose mothers had an active phase of labor compared with infants whose mothers had no labor or did not progress beyond the latent phase. Infants who had early periventricular-intraventricular hemorrhage (≤1 hour of age) also had a higher frequency of progression of hemorrhage.

CONCLUSIONS: Cesarean section before the active phase of labor does not change the overall frequency of hemorrhage but results in a lower frequency of progression to grade 3 or 4 hemorrhage. We do not feel that these data support performing more cesarean sections for preterm delivery as a method of preventing progression of periventricular-intraventricular hemorrhage in the preterm infant. (Am J Obstet Gynecol 1992;166:1091-101.)

Key words: Periventricular-intraventricular hemorrhage, active-phase labor, time of hemorrhage, cesarean section, progression

The relationship of route of delivery to the pathogenesis of periventricular-intraventricular hemorrhage in the neonate is much less controversial than before. The preponderance of recent studies has shown that the frequency is independent of the mode of delivery.[1-5] A recently published large multicenter study[6] involving 1765 infants with birth weights of ≤1500 gm confirmed these observations. It found that cesarean section did not lower the risk of mortality or periventricular-intraventricular hemorrhage.

However, the time when periventricular-intraventricular hemorrhage occurs may be affected by active labor. A preliminary report from our institution suggested that infants whose mothers experienced the active phase of labor, regardless of the route of delivery, had an increased frequency of periventricular-intraventricular hemorrhage in the first hour of life as compared with the infants of women delivered by cesarean section before active-phase labor.[7] We previously reported that in infants delivered by cesarean section before the active phase of labor periventricular-intraventricular hemorrhage developed in 7.7% at ≤1 hour after delivery compared with 42.9% of infants whose

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Table I. Periventricular-intraventricular hemorrhage by birth weight distribution

Birth weight (gm)	*Stage of labor*			*Periventricular-intraventricular hemorrhage*		
	Active	*Latent*	*None*	*Yes*	*No*	*Periventricular-intraventricular hemorrhage (%)*
500-750	4	3	6	10	3	76.9
751-1000	6	5	9	11	9	55.0
1001-1250	7	7	10	12	12	50.0
1251-1500	4	13	12	6	23	20.7
1501-1750	4	4	6	5	9	35.7
>1750	2	1	3	1	5	16.6
TOTAL	27	33	46	45	61	42.4

Table II. Characteristics of mother and infants in cesarean section deliveries

	Cesarean section (n = 106)	
	No.	*%*
Preterm labor	56	51
Antenatal steroids	44	41
Active phase labor	27	25
Oxytocin	21	20
Tocolytic agents	35	33
Vertex presentation	60	67
Resuscitation	66	62
Black	69	65
White	37	35
Male	46	42
Female	60	58
Left delivery room intubated	53	50
Birth weight (gm, mean ± SD)	1210 ± 352	
Gestational age (wk, mean ± SD)	29.5 ± 2.7	
pH at admission (mean ± SD)	7.30 ± 0.08	
Base deficit at admission (mmol/L, mean ± SD)	−3.2 ± 3.3	
Hematocrit at admission (%, mean ± SD)	46 ± 7	

mothers were in active-phase labor ($p = 0.006$). However, by day 7 of life, the frequency of periventricular-intraventricular hemorrhage in infants delivered by cesarean section before active-phase labor was 38.5%, which was not significantly different from the 52 4% found in the infants whose mothers experienced the active phase of labor.

Interestingly, of the infants who experienced the active phase of labor and had periventricular-intraventricular hemorrhage, 18 of 33 (54.5%) had progression of hemorrhage to grade 3 or 4 compared with 0 of 8 in the no-active-phase labor group. We were impressed with how similar the infants whose mothers had cesarean section after entering the active phase of labor were to the infants delivered vaginally, with regard to both the onset and the progression of hemorrhage. To further examine the role of active-phase labor rather than route of delivery, we decided to study the preterm infants delivered by cesarean section that were part of a continuing study at our institution. Our hypothesis was that infants with periventricular-intraventricular hemorrhage delivered by cesarean section after the active phase of labor would have the same findings with regard to timing and progression of hemorrhage as would previously studied infants that were delivered vaginally. We also expected a higher frequency of early hemorrhage (≤1 hour of age), a similar overall frequency of hemorrhage at 7 days of life, and a higher frequency of progression to grade 3 or 4 hemorrhage in the infants who have cesarean section after active-phase labor compared with infants delivered by cesarean section before active-phase labor. We report a prospective study that includes serial ultrasonographic head scans of the infants, with the initial scan performed in the first few minutes of life, to examine the role of active-phase labor and development of periventricular-intraventricular hemorrhage. This study reports on all the infants delivered by cesarean section in this study.

Material and methods

This study was carried out at the Regional Medical Center, Memphis, after review and approval by the Institutional Review Board of the University of Tennessee, Memphis. Informed consent was obtained from the mother before delivery of the infant. All women,

Table III. Characteristics of infants with and without periventricular-intraventricular hemorrhage

	Periventricular-intraventricular hemorrhage				
	Yes (n = 45)		*No (n = 61)*		
	No.	*%*	*No.*	*%*	*p Value*
Mother's race					
White	16	43.2	21	56.8	NS
Black	29	42.0	40	58.0	NS
Infant's sex					
Male	25	54.3	21	45.7	NS
Female	20	33.3	40	66.7	0.049
Resuscitation at delivery	35	53.0	31	47.0	NS
Antenatal steroids					
Yes	16	36.8	28	63.2	NS
No	29	46.7	33	53.3	NS
Tocolytic agents					
Yes	18	51.4	17	48.6	NS
No	28	39.4	43	60.6	NS
Mother's age (yr, mean ± SD)*		22 ± 3.6		23 ± 4.8	NS
Mean birth weight (gm, mean ± SD)*		1072 ± 336		1313 ± 330	0.000
Gestational age (wk, mean ± SD)*		28.4 ± 2.8		30.3 ± 2.4	0.000
Cord blood pH (mean ± SD)		7.32 ± 0.5		7.31 ± 0.07	NS
Cord blood P_{CO_2} (mean Hg, mean ± SD)		46 ± 8.0		44 ± 8.0	NS
Cord blood P_{O_2} (mean Hg, mean ± SD)		37 ± 23		34 ± 34	NS
Cord blood base deficit (mmol/L, mean ± SD)		−1.97 ± 2.3		−2.12 ± 3.4	NS
Cord blood hematocrit (mmol/L, mean ± SD)		40 ± 8.0*		47 ± 6.0†	0.007

NS, Not significant.
*n = 19.
†n = 26.

irrespective of age or parity, admitted to the labor and delivery area from January 1986 to June 1988 with an ultrasonographically estimated fetal weight ≤1750 gm were screened for entry into the study. Medical and obstetric risk factors were recorded. The length of labor, frequency of uterine contractions, length of the active phase of labor, and length of the second stage of labor were recorded. The active phase was arbitrarily considered to begin when the cervix dilated to 4 cm. For women in preterm labor or with the diagnosis of preterm rupture of the membranes, the active phase was considered to start at the last time the cervix was found to be 4 cm dilated before progression into the active phase of labor. When appropriate, women in preterm labor were treated with tocolytic agents.

Antenatal steroids were given at the discretion of the attending physician. Women were allowed to deliver vaginally unless there were obstetric or maternal indications for cesarean section. Outlet forceps were used during vaginal delivery at the discretion of the attending staff. Obstetric management was carried out under the supervision of the maternal-fetal medicine attending staff.

The pediatric team members were present at delivery and were responsible for neonatal resuscitation, stabilization, and assignment of the Apgar scores. As soon as the baby's respiration or ventilation was established, the initial head ultrasonographic scan of the neonate was performed. This procedure was done by experienced personnel assigned to the research project while the neonate's primary caretakers continued with the medical management of the neonate. After stabilization, the infants were transported to the neonatal intensive care unit for further assessment and treatment.

Findings of periventricular-intraventricular hemorrhage were graded according to the criteria of Papile et al.[8] Grade 1 refers to hemorrhage confined to the germinal layer, grade 2 is intraventricular hemorrhage, grade 3 is intraventricular hemorrhage with ventricular dilatation, and grade 4 includes associated parenchymal hemorrhage or extension. Ultrasonographic images were videotaped and later read blinded by one of the investigators (H.L.M.) without any clinical data.

Infants who weighed ≤1500 gm underwent ultrasonographic head scans at delivery and at 1, 6, 12, and 24 hours of age and then daily for the first 7 days of

Table IV. Age at initial detection of periventricular-intraventricular hemorrhage in infants of women who had cesarean section

Time after birth (hr)	*Periventricular-intraventricular hemorrhage*		*Cumulative total (%)*
	No.	*%*	
<1	9	20.0	20
1	3	6.6	26.6
6	2	4.4	31.1
12	3	6.6	37.7
24	4	8.8	46.6
48	10	22.2	68.8
72	11	24.4	93.3
>72	3	6.6	100.0

Table V. Timing of periventricular-intraventricular hemorrhage in infants of women who had cesarean section

Time (hr)	*Active-phase labor (cervix ≥4 cm) (n = 14)*		*Latent-phase labor (cervix <4 cm) (n = 12)*		*No labor (n = 19)*	
	No.	*Cumulative total (%)*	*No.*	*Cumulative total (%)*	*No.*	*Cumulative total (%)*
<1	3	21.4	3	25.0	3	15.8
1	2	35.7	1	33.3	0	15.8
6	0	35.7	1	41.7	1	21.0
12	0	35.7	1	50.0	2	31.6
24	1	42.9	3	75.0	0	31.6
48	2	66.6	2	91.6	6	63.1
72	5	92.9	1	100.0	5	89.5
>72	1	100.0			1	100.0

life. Infants who weighed >1500 gm had ultrasonographic head scans at the same time interval for the first 24 hours of life and then had a head scan at 7 days of life. Babies who died in the first week also had a head scan procedure immediately post mortem.

Approximately 4000 maternal and neonatal variables were collected per mother-baby pair. These variables were recorded on precoded forms, reviewed, corrected, and entered into the mainframe computer, Vax 8800 (Digital Equipment, Inc, Maynard, Mass.). The Oracle Database Management System (Oracle Corporation, Belmont, Calif.) was used. Extensive edit checks were programmed and run after data entry, and errors were corrected accordingly.

The effect of labor in women who underwent cesarean section with regard to periventricular-intraventricular hemorrhage or progression to grade 3 or 4 periventricular-intraventricular hemorrhage in the neonate was examined. Hemorrhages detected within 1 hour of age were arbitrarily defined as early hemorrhage and those detected later than 1 hour of age were referred to as late hemorrhage.

Maternal obstetric and neonatal factors were then analyzed for their relationship to periventricular-intraventricular hemorrhage. Continuous variables were compared by one-way analysis of variance among those infants with no hemorrhage or periventricular-intraventricular hemorrhage. Categoric variables were compared by χ^2 analysis and Fisher's exact test when appropriate. A p value of <0.05 was considered significant.

Results

We studied 106 infants of 85 women who were delivered by cesarean section. There were 16 pairs of twins, with the first twin of one woman not enrolled in the study. There was no significant difference in the distribution of infants in any of the subgroups by weight among the three groups (Table I). Periventricular-intraventricular hemorrhage occurred in 45 of the 106 infants (42.4%) and in 39 of 86 infants (45.3%) with birth weights ≤1500 gm. Table II describes the entire group of mothers who had cesarean section and their infants. The mean birth weight was 1210 ± 351 gm and the mean gestational age was 29.5 ± 2.7 weeks.

Forty-one percent of the infants were exposed to antenatal steroids and 33% to tocolytic agents. Fifty percent of the infants left the delivery room intubated. Table III categorizes the infants as to whether periventricular-intraventricular hemorrhage developed. The infants with development of periventricular-intraventricular hemorrhage were lighter (1072 ± 336 vs

Table VI. Maternal diagnosis or primary indication for cesarean section

Diagnosis	*Birth weight (gm, mean ± SD)*	*Periventricular-intranventricular hemorrhage*		*Proportion with periventricular-intraventricular hemorrhage (%)*
		Yes	*No*	
Breech presentation*	1191 ± 424	17	19	47.2
Hypertension or pregnancy-induced hypertension	1299 ± 299	7	21	25.0
Fetal distress	1098 ± 267	9	8	52.9
Abruptio placentae	1134 ± 339	2	6	25.0
Placenta previa	976 ± 189	2	0	100.0
Chorioamnionitis	1320 ± 339	4	2	66.6
Other	1264 ± 352	4	5	44.4

*Five women with breech presentation had another diagnosis or indication for cesarean section.

Table VII. Periventricular hemorrhage in twins delivered by cesarean section

	*Twin A (n = 15)**	*Twin B (n = 16)*
Birth weight (gm, mean ± SD)	1268 ± 295	1110 ± 266
Birth weight with periventricular-intraventricular hemorrhage (gm, mean ± SD)	1137 ± 158	928 ± 186
Periventricular-intraventricular hemorrhage		
<4 cm	2/9	4/9
≥4 cm	2/6	3/7
Total	4/15	7/16
Progression to grade 3 or 4 hemorrhage	2/4	2/7

*One first twin was not enrolled in the study.

1313 ± 330 gm, $p = 0.000$) and were less advanced in gestation (28.4 ± 2.8 vs 30.3 ± 2.4 weeks, $p = 0.000$), when compared with the infants without development of hemorrhage. The frequency of periventricular-intraventricular hemorrhage was similar in infants whether they did or did not receive antenatal steroids or tocolytics. Cord blood hematocrits were also significantly lower in the infants with development of periventricular-intraventricular hemorrhage ($p = 0.007$). A lower frequency of hemorrhage occurred in female (33.3%) as compared with male (54.3%, $p = 0.049$) infants. Table IV lists the age at which the periventricular-intraventricular hemorrhage was first detected. Ninety percent of the entire group of infants had the initial head ultrasonographic examinations performed in the first 20 minutes of life. Only 3.9% had the initial examination performed >30 minutes from birth. Periventricular-intraventricular hemorrhage had already occurred at the time of the initial head ultrasonography in 20.0% of all the infants who had periventricular-intraventricular hemorrhage. By 12 hours of life, another 17.7% of the infants had had periventricular-intraventricular hemorrhage. However, in 24 (53.4%) of the 45 infants with hemorrhage, it occurred at ≥48 hours of life. The hemorrhage occurred after 72 hours of life in only 6.6% of these infants. Table V shows the timing of onset of the hemorrhage in the groups of active-phase labor (cervical dilatation ≥4 cm), latent-phase labor (cervical dilatation <4 cm), and no labor. There were no significant differences in the time that the periventricular-intraventricular hemorrhage developed between the three groups.

Table VI reports the primary indication or diagnosis of the women who had cesarean section and the frequency of periventricular-intraventricular hemorrhage. The primary indication for cesarean section was breech presentation in 36 of 106 infants (34.0%). Another 28 (32.1%) mothers had pregnancy-induced hypertension, and 17 infants (16.0%) were delivered by cesarean section because of fetal distress. There were no significant differences in the frequency of periventricular-intraventricular hemorrhage with regard to maternal diagnosis.

The frequency of periventricular-intraventricular hemorrhage is compared between twins in Table VII. The frequency of periventricular-intraventricular hemorrhage and progression to grade 3 or 4 hemorrhage was not significantly different between the first and second twins.

The effect of when during labor the mother had cesarean section is shown in Tables VIIIA and VIIIB. The overall frequency of hemorrhage, the frequency of early or late hemorrhage, and the initial frequency of grade 3 or 4 hemorrhage were similar among the three groups. However, progression of hemorrhage, progression to grade 3 or 4 hemorrhage, and total fre-

Table VIIIA. Periventricular-intraventricular hemorrhage compared with time in labor mothers had cesarean sections

	Active-phase labor (n = 27)		*Latent-phase labor (n = 33)*		*No labor (n = 46)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*p Value*
Birth weight (gm, mean ± SD)	1192	387	1208	387	1208	294	NS
Periventricular-intraventricular hemorrhage							
≤1 hr of age	5	18.5	4	12.1	3	6.5	NS
>1 hr of age*	9	40.9	8	27.5	16	37.2	NS
Total	14	51.9	12	36.3	19	41.3	NS

NS, Not significant.

*Excludes infants in whom periventricular-intraventricular hemorrhage developed at ≤1 hour of age.

Table VIIIB. Progression of hemorrhage

	Active-phase labor and hemorrhage (n = 14)		*Latent-phase labor and hemorrhage (n = 12)*		*No labor and hemorrhage (n = 19)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*p Value*
Progression of hemorrhage within grade to higher grade	9	64.3	3	25.0	1	5.3	0.001
Grade 3 or 4 hemorrhage							
Initial	2	14.3	1	8.3	4	21.1	NS
Progression*	8	66.7	1	9.9	1	6.7	0.022
TOTAL	10	71.4	2	16.6	5	26.3	0.006

NS, Not significant.

*Excludes infants in whom periventricular-intraventricular hemorrhage developed at ≤1 hour of age.

quency of grade 3 or 4 hemorrhage were significantly higher in the active-phase labor group as compared with the latent-phase and no-labor groups.

Table IX gives the initial and final grades of periventricular-intraventricular hemorrhage of the two groups. Excluding infants whose initial hemorrhage was grade 3 or 4, only 2 of 27 infants (7.7%) in the no-active-phase labor group had progression to grade 3 or 4 hemorrhage compared with 8 of 12 (66.7%) in the active-phase labor group ($p = 0.000$).

Table X examines the mean birth weight of the groups compared with early or late hemorrhage and progression of hemorrhage. The infants who did not have periventricular-intraventricular hemorrhage had significantly higher birth weights than did the infants who had periventricular-intraventricular hemorrhage. The infants who had progression of hemorrhage were more likely to have lower weights.

Of additional interest is the effect of early hemorrhage on the progression of periventricular-intraventricular hemorrhage. Six of the 12 infants (50%) who had development of periventricular-intraventricular hemorrhage ≤1 hour of age had hemorrhage that progressed one or more grades, as compared with 6 of 33 infants (18.2%) who developed periventricular-intraventricular hemorrhage >1 hour of age ($p = 0.043$).

Comment

As expected, the infants with development of periventricular-intraventricular hemorrhage weighed less and were less advanced in gestation as compared with the infants in whom periventricular-intraventricular hemorrhage did not develop. In addition, fewer female infants experienced periventricular-intraventricular hemorrhage. There were no differences in frequency by maternal diagnosis, breech presentation, twins, tocolytic therapy, or antenatal steroids. A similar percentage of infants received antenatal steroids in the active-labor and in the no-labor groups. As expected, the number of infants who received tocolytic therapy was less for those whose mothers were not in active labor (18 of 78 infants, 23.1%) than for those whose mothers were in the active phase of labor (17 of 27 infants, 62.9%, $p < 0.001$). Cord blood gas values were similar in the hemorrhage and no-hemorrhage groups. Cord blood hematocrit values were significantly lower in the infants with periventricular-intraventricular hemorrhage. In infants with early periventricular-in-

Table IX. Initial and final grades of infants with development of periventricular-intraventricular hemorrhage

	Cesarean section			
	Active-phase labor (n = 14)		*No-active-phase labor (n = 31)*	
Severity of hemorrhage	*Initial grade*	*Final grade*	*Initial grade*	*Final grade*
Grade 1	5	1	8	7
Grade 2	7	3	18	17
Grade 3	1	8	3	4
Grade 4	1	2	2	3

Table X. Mean birth weights and time of hemorrhage

		Periventricular-intraventricular hemorrhage				
		≤1 hr of age		*>1 hr of age*		
	No hemorrhage	*Stable*	*Progression*	*Stable*	*Progression*	*Total*
No labor	1348 ± 343 (*n* = 27)	1085 ± 546 (*n* = 3)		1015 ± 330 (*n* = 15)	942 (*n* = 1)	1213 ± 377 (*n* = 46)
Latent labor	1202 ± 254 (*n* = 21)	1712 ± 267 (*n* = 3)	652 (*n* = 1)	1176 ± 141 (*n* = 7)	998 (*n* = 1)	1220 ± 249 (*n* = 33)
Active labor	1415 ± 378 (*n* = 13)		945 ± 249 (*n* = 5)	1083 ± 346 (*n* = 5)	911 ± 188 (*n* = 4)	1192 ± 386 (*n* = 27)

traventricular hemorrhage the low cord blood hematocrit may be due to the hemorrhage itself. However, cerebral blood flow increases with a decreasing hematocrit[9] so that a low cord hematocrit may be a predisposing factor to the development of periventricular-intraventricular hemorrhage.[10] We concur with others[2, 5] and remain surprised at the small role obstetric factors appear to have with regard to the development of periventricular-intraventricular hemorrhage. In this article we focused on when it occurred and the progression and overall frequency of grade 3 or 4 hemorrhage. The time when periventricular-intraventricular hemorrhage occurs is most important with regard to the development of therapeutic interventions. If a large percentage of hemorrhages are present at delivery, the intervention, such as drug administration to the mother, would need to start before delivery. On the other hand, if only a small percentage of hemorrhages are present at birth, then intervention directed at the neonate would be appropriate. Additionally, we need to study the relationship of the time when hemorrhage occurs with regard to progression of hemorrhage.

Severe hemorrhage (grade 3 or 4) has a much higher mortality and a much higher frequency of neurologic deficit associated with it. Previous reports of infants who survived with grade 3 or 4 hemorrhage indicated a 50% to 75% frequency of neurologic deficits[8, 11] compared with a 15% frequency in the infants who survived grade 1 or 2 hemorrhage.[12] Although some recent reports are more optimistic, grade 3 or 4 hemorrhages are still associated with a high percentage of neurologic deficits in the infants.[13, 14]

The frequency of early hemorrhage and overall frequency of periventricular-intraventricular hemorrhage was not different in the infants whose mothers were in active-phase labor as compared with infants whose mothers were not in labor. We were surprised to find that there were no significant differences in the time that the three groups developed periventricular-intraventricular hemorrhage. From our previous report,[7] we expected that the infants whose mothers were in active labor at the time of cesarean section would have a higher percentage of early hemorrhage, less late hemorrhage, and the overall frequency of hemorrhage the same when compared with the other two groups. However, the frequency of early hemorrhage varied from three of 46 infants (6.5%) in the no-labor group to five of 27 infants (18.5%) in the active-phase labor group. Additionally, among all infants with ultimate development of periventricular-intraventricular hemorrhage, it developed in the first hour of life in 15.8% of the no-labor group as compared with 35.7% in the active-phase labor group. We contrasted these findings to those of our previous report of 53 infants who were delivered by the vaginal route with a mean birth weight of 1298 gm; periventricular-intraventricular hemorrhage developed in 21 of 53 infants (39.6%) in the first hour of life. Additionally, of all the infants with periventricular-intraventricular hemorrhage, 75% had their hemorrhage within the first hour of life. Although

Table XI. Frequency of grade 3 or 4 hemorrhage

Series	No.	Birth weight (gm)	Periventricular hemorrhage			
					Grade 3 or 4 hemorrhage	
			No.	%	No.	%
Wallin et al.[16]	463	≤1500	128	28	52	45
Morales and Koerten[17]	427	≤1500	213	45	85	40
Philip et al.[18]	643	≤1500	206	34	79	38
Rayburn et al.[2]	303	≤1500	103	30	58	56
TOTAL	1813		650	36	274	42

Table XII. Maternal diagnosis associated with immature delivery (n = 321)

	No.	%
Premature rupture of membranes	99	30.8
Preterm labor	82	25.5
Abruptio placentae or placenta previa	65	20.2
Preeclampsia or eclampsia	44	13.7
Incompetent cervix	29	9.0
Other	2	0.06

From Amon E, Anderson DG, Sibai BM, Mabie WC. Factors responsible for preterm delivery of the immature newborn infant (≤1000 gm). Am J Obstet Gynecol 1987;156:1143-8.

not specifically investigated in this study, these findings suggest our previous conclusions that cesarean section before the onset of active-phase labor is protective for early periventricular-intraventricular hemorrhage but not for overall frequency of hemorrhage. Additionally, vaginal delivery accelerates the time when hemorrhage occurs but does not affect the overall frequency of hemorrhage. Active-phase labor may well fit between these two points on a continuum with some effect on the frequency of early hemorrhage but certainly not to the same degree as vaginal delivery.

In 17 of the 45 infants (37.7%) the final grade of hemorrhage was 3 or 4. The initial hemorrhage in 7 of the 17 infants was grade 3 or 4, and 10 other infants had progression of hemorrhage to grade 3 or 4. Seven (22.5%) of the 31 infants who were delivered before the active phase of labor (latent-phase and no-labor groups) had periventricular-intraventricular hemorrhage grade 3 or 4 hemorrhage. The initial hemorrhage in five of the seven infants was grade 3 or 4. Only two of 26 infants (excluding the five infants whose initial hemorrhage was grade 3 or 4) had progression of hemorrhages to grade 3 or 4. In contrast, 10 of the 14 infants (71.4%) who experienced active-phase labor had grade 3 or 4 hemorrhage (p = 0.006). Two infants had initial hemorrhages that were grade 3 or 4, whereas eight other infants had progression of hemorrhage to grade 3 or 4 (p = 0.022). A recent study was reported on 49 infants who had a head ultrasonographic scan done immediately after birth or after admission to the neonatal intensive care unit.[15] Twenty of the 49 infants were delivered by cesarean section. Of the 20 infants with periventricular-intraventricular hemorrhage, 6 (30%) had hemorrhage detected at the initial head ultrasonographic scan. Thirteen of the 20 (65%) had progression of hemorrhage. In four large studies (Table XI), 42% of the infants with periventricular-intraventricular hemorrhage had grade 3 or 4 hemorrhage.[2, 16-18] Our frequency of 37.5% of grade 3 or 4 hemorrhage is similar to the incidence in these studies. However, the frequency of severe hemorrhage of 22.5% in the infants who were not in active-phase labor was half of that reported in these large series. We feel that absence of the active phase of labor is protective against progression to severe hemorrhage. It is of great interest that 6 of 12 infants (50%) with periventricular-intraventricular hemorrhage that developed at ≤1 hour of life had progression of hemorrhage compared with only 6 of 33 infants in whom it developed at >1 hour of age (p = 0.043). If this holds true, then any factor or factors that cause periventricular-intraventricular hemorrhage during labor, at the time of delivery, or in the first hour of life would also cause an increased frequency of progression of hemorrhage.

If cesarean section before the active phase of labor prevents progression to severe periventricular-intraventricular hemorrhage, should more cesarean sections be performed in the preterm infants to prevent progression to severe hemorrhage? The answer is no. Table XII gives the indication for delivery of 321 liveborn infants with birth weights <1000 gm unrelated to this study. We examined these indicators for the practicality of cesarean section at ≤4 cm. In women who undergo cesarean section because of incompetent cervix, the cervix is usually dilated to ≥4 cm at the time of admission. Most women with preeclampsia or eclampsia ≤1500 gm are delivered by cesarean section. Essentially, all immature infants whose mothers have placenta previa or abruptio placentae are delivered by

cesarean section. No one would advocate cesarean section in women with preterm labor before a cervical dilatation of 4 cm in an attempt to decrease the progression of periventricular-intraventricular hemorrhage after delivery.

Premature rupture of members is the only diagnosis that would lend itself to changes. However, to plan preterm delivery and expose the neonate to serious problems of prematurity in the hope of preventing a single serious problem would seem unwarranted.

We do not feel that our study supports any change in the route of delivery of the preterm infant.

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Discussion

DR. JAMES A. LOW, Kingston, Ontario, Canada (Fellow). Recent progress in our knowledge of perinatal brain damage and its underlying pathogenic mechanisms has been due, in part, to the increasing use of imaging techniques to monitor the development and evolution of pathologic lesions of the brain in surviving newborn infants. This report was designed to demonstrate that active labor in preterm pregnancy with a fetus <1750 gm will increase the frequency, timing, and severity of periventricular-intraventricular hemorrhage in the newborn. Analysis of the data derived from 106 preterm newborns delivered by cesarean section has demonstrated no significant difference in either the frequency or the timing of periventricular-intraventricular hemorrhage in the active-labor group in relation to the no-active-labor group. Therefore the authors have focused their attention on the severity of the periventricular-intraventricular hemorrhage as expressed by the final grade during the week after delivery. Analysis indicates that the incidence of grades 3 and 4 periventricular-intraventricular hemorrhage in the active-labor group is significantly greater than in the no-active-labor group (Table IX). However, three issues should be considered before this interpretation is accepted.

Active labor is the central question in this study. The discrimination between the no-labor group ($n = 46$) and the active-labor group ($n = 27$) is clear. However, the latent-labor group ($n = 33$) could represent a confounding factor. Active labor was defined as beginning when the cervix dilated to 4 cms. If we assume there are multiple observers, have these observations been consistent throughout the study? Do the authors have data with regard to the duration of uterine activity or the total amount of uterine work in the patients of the latent-labor and active-labor groups? For example, in respect to the stress to the fetus, is there a difference between a patient with 8 hours of latent labor resulting in cervical dilatation of 3 cm and a patient with 3 hours of active labor resulting in cervical dilatation of 5 cm? Would the interpretation of the association of grades 3 and 4 lesions change if the latent-labor group was either excluded or transferred to the active-labor group?

The possibility of confounding clinical variables must be considered. The authors state that approximately 4000 maternal and neonatal variables were collected per mother and baby pair. Maternal obstetric and neonatal variables were then analyzed for their relationship to periventricular-intraventricular hemorrhage. The association of birth weight, newborn sex, and cord he-

matocrit with periventricular-intraventricular hemorrhage is noted in Table III. Should it be assumed these represent the only significant clinical variables associated with periventricular-intraventricular hemorrhage? Newborn complications in particular are important because the final grade of periventricular-intraventricular hemorrhage occurred during the week after delivery. Associations with periventricular-intraventricular hemorrhage have been demonstrated for newborn respiratory complications[1] and newborn hypotension.[2,3] Thus the possible associations of newborn respiratory distress syndrome, pneumothorax, newborn hypoxemia, and newborn hypotension, and hypertension should be examined. A logistic regression analysis to determine the association of active labor, birth weight, and any relevant newborn complications to periventricular-intraventricular hemorrhage would help to justify the conclusion that there is a significant association between active labor and the final grade of the periventricular-intraventricular hemorrhage.

The authors are to be commended for the carefully scheduled series of head ultrasonographic scans beginning during the hour after delivery. This protocol has provided documentation of the time of the first ultrasonographic evidence of periventricular-intraventricular hemorrhage in each child, within the limits of the sensitivity of cranial ultrasonographic examinations.

The final grade of the periventricular-intraventricular hemorrhage was defined according to the criteria of Papile et al. The significant observation identified by the authors was an increased incidence of grades 3 and 4 periventricular-intraventricular hemorrhage in the active-labor group. However, examination of the data in Table IX indicates that the frequency of grade 4 is of the same order in the active-labor and no-active-labor groups. The contiguous hyperechoic lesion in the parenchyma of the brain identified by ultrasonographic examination (classified as grade 4) represents in most cases hemorrhagic periventricular leukomalacia rather than a direct extension of a germinal matrix hemorrhage.[4] Were there other ultrasonographically demonstrated parenchymal lesions in these newborns? If so, these parenchymal lesions should be considered as a separate group. Thus the association between active labor and the severity of periventricular-intraventricular hemorrhage is based on an increased number of grade 3 in relation to grade 2 lesions in the active-labor group compared with the no-active-labor group. Is the degree of ventriculomegaly that discriminates the grade 3 from the grade 2 lesions substantive?

The significance of a positive finding would be an increase in elective cesarean sections in the delivery of preterm pregnancies. The authors concluded that more cesarean sections should not be performed to prevent periventricular-intraventricular hemorrhage because the current indications in these patients would not justify such intervention. In view of the data presented in this study, would it not be more appropriate to conclude that the evidence for severe hemorrhage because of active labor is not sufficiently compelling to justify such aggressive intervention?

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Dr. Thomas H. Kirschbaum, Bronx, New York. Dr. Anderson, it seems to me you presented a series of women in preterm labor, some of them anticipating vaginal delivery, who had complications of labor sufficiently compelling to result in cesarean section. How do you separate the effect of the indications for cesarean section from the impact of the procedure itself in terms of the differences you noted?

Dr. Valerie Parisi, Houston, Texas. Dr. Anderson, on the neonatal side of the coin, if you eliminate the babies who had hemorrhage diagnosed within the first hour of life and look at the rest of the babies, was there any effect of maximum ventilator settings or time on the ventilator in the incidence or progression of hemorrhage in those babies?

Dr. Mortimer Rosen, New York, New York. I have one question. There is a suggestion that the lowest-birth-weight babies were in the vaginal delivery group. If one looks at individual birth weights, not at 1700 gm but going down to 500 and 600 gm, in clinical practice it is more common for that baby to be delivered vaginally, one, because it comes out more quickly, and two, because the obstetrician doesn't want to do a cesarean. Could that have confounded your results?

Dr. Paul G. McDonough, Augusta, Georgia. A statistical estimation of the difference between normal and abnormal afforded by the test system would facilitate data interpretation. Do you have some measure of ultrasonographic intraobserver and interobserver variability in attempting to distinguish between normal and the various grades of hemorrhage?

Dr. Steven G. Gabbe, Columbus, Ohio. Dr. Anderson, did you screen mothers for a history of anticoagulant use? Particularly, did you find out if these mothers had used aspirin?

Dr. Anderson (Closing). With regard first to the questions that Dr. Low asked, and I guess the most important one concerns the indications we now consider with regard to cesarean section, that we shouldn't do more cesarean sections or whether there is sufficient evidence that active-phase labor leads to severe hemorrhage.

I think that there is reasonable evidence, although

the numbers are small, that in either vaginal delivery or cesarean section, if the infants have experienced active labor, there is a higher incidence of grade 3 or 4 hemorrhage.

Although not mentioned in this presentation with regard to neonatal complications, if one group of infants was sicker than the other, it was the infants who were not subjected to active labor. In the infants who did not undergo active labor, 14 of the 79 infants died. In the active-labor group there was one death. This didn't quite reach clinical significance, but if there was any skewing of the two groups, the infants not subjected to active labor were the sicker of the two groups.

With regard to latent labor and what would happen if we lumped the groups on either side, certainly, if we take out the latent-phase group, there is still a significant difference comparing just the active-phase and the no-labor groups. If we put the latent-phase group in with the active-phase labor group, it is also still significant. I think that the latent-phase group is interesting, but they certainly are much more like the women who have not had labor as compared with the active-phase labor group. With regard to the results of severe hemorrhage in our study, there were more in the grade 3 than the grade 4 category and what does this mean? I think in most of the studies, when the criteria of Papile et al. are used and the number of survivors is examined, there are likely to be more in the grade 3 than the grade 4 category.

Dr. Kirschbaum asked whether the indication for cesarean section had an impact on the study. Was the severity of the condition the reason for cesarean section? Again, because the less-sick infants were the active-phase group, this should not have affected the outcomes that we found.

Dr. Parisi asked if we looked at what happened in the neonatal course among the three groups. Among the first 150 infants studied, delivered both vaginally and by cesarean section, if the infants who had hemorrhage within the first hour of life are taken out, certainly there is no difference in major neonatal complications among the infants who did or did not have intraventricular hemorrhage.

A question was asked about all the other variables. We have now looked at all 230 infants we studied and evaluated obstetric variables with regard to frequency of hemorrhage; basically we have found that only cord blood hematocrit, male gender, and active-phase labor were the only variables that are associated with early hemorrhage.

Dr. Rosen asked about the birth weights within the three groups. At least in this study, by 250 gm increments, there were no significant differences among the three groups with regard to birth weights. In the previous study there also were significant differences by 250 gm increments. So there was not a significantly higher incidence of very-low-birth-weight infants in the group delivered vaginally.

I'm not sure I exactly understand the question about ultrasonography other than to say the clinicians taking care of the patient looked at the results and used them for clinical management. All the ultrasonographic scans were read in a blinded manner by one of the participants in the study with absolutely no clinical data about the infants.

In regard to Dr. Gabbe's question about aspirin, yes, we did screen for aspirin in this study and got a lower instance than we would suspect. I've forgotten the exact number, but there were no substantial differences among the three groups with regard to mothers ingesting aspirin.

Leadership qualities of obstetrics and gynecology department chairmen of United States medical schools

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OBJECTIVE: The purpose of this study is to examine leadership qualities, preparation for leadership, and perceptions of skills needed for academic leaders in obstetrics and gynecology.
STUDY DESIGN: Chairmen and acting chairmen of the 122 United States medical schools with residency training programs in obstetrics and gynecology were surveyed about the stated objectives of the study. Differences in proportions were analyzed by means of the χ^2 test; the Student *t* test was used to compare mean ranks.
RESULTS: Information was obtained from 98 (80.3%) of those surveyed. Interpersonal skills and knowledge of the specialty were identified as most important qualities leading to appointment; financial management skills were identified as most frequently lacking. Having a mentor prior to appointment was found to be associated with several characteristics. Those chairmen who had mentors were more likely to have these characteristics: (1) to have completed a subspecialty fellowship, (2) to command a larger departmental budget (>$4 million), (3) to have been a board examiner before appointment, and (4) to have received support in obtaining their appointment from recognized leaders in the specialty.
CONCLUSION: Current academic leaders in obstetrics and gynecology must identify and prepare protégés (protégées) for future leadership roles. (Am J Obstet Gynecol 1992;166:1102-16.)

Key words: Leadership skills, mentor

Development and selection of corporate leaders is a frequent topic in the management literature.[1-4] However, few studies are published on the development and selection of leaders in medical academia.[5-8] Prentice wrote, "Attempts to analyze leadership tend to fail because the would-be analyst misconceives the task. Leadership is usually not studied at all. Popularity, power, showmanship, and wisdom in long-range planning are studied instead. Some leaders have these things, but they are not of the essence of leadership. The leader's *unique* achievement is a human and social one which stems from understanding fellow workers and the relationship of their individual goals to the group that must be carried out."[9]

The tasks that confront chairmen* of clinical departments of medical schools are continually increasing and changing.[10] Chairmen are expected to direct undergraduate, residency, and subspecialty fellowship educational programs, lead basic and clinical research programs supported by extramural funding, preside over a large clinical practice that generates funds for faculty support and institutional survival, serve on institutional committees, maintain a national presence and reputation, and manage the personnel and fiscal affairs of the department. Moreover, clinical departmental chairmen must stay abreast of federal and state health care reimbursement schemes, accreditation requirements for educational programs and research laboratories, and patient admission policies of hospitals and ambulatory settings.

No one can be fully prepared for such a multifaceted position. Yet most men and women who are selected as chairmen of medical school departments of obstetrics and gynecology thrive in the position and serve for >5 years. The purpose of this study is to examine leadership qualities, preparation for leadership, and perceptions of skills needed for academic leaders in obstetrics and gynecology.

Material and methods

Information was gathered by survey (see appendix) of current and acting chairmen of departments of obstetrics and gynecology of United States medical schools with residency training programs. Chairmen were identified as those listed in the *AAMC Directory of American Medical Education, 1990-91*.[11] Surveys were mailed to each current and acting department chairman (N = 122) with a cover letter explaining the purpose of the survey and requesting that the questionnaire be returned within 21 days of receipt.

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Items that were scaled according to rank were assigned a value of 1 to the most important item and a value that constituted the number of items to be ranked as the least important (i.e., seven items to be ranked so a value of 7 indicates least importance). When a rank was not assigned at all, the value of least importance was inserted for calculation of mean ranks.

Differences in proportions were analyzed with the χ^2 test.[14] The Student t test was used to compare mean ranks.[15] A value of $p \leq 0.05$ was considered to be statistically significant. All data analysis was performed with SAS statistical software (SAS Institute Inc., Cary, N.C.).

Results

Surveys were returned from 98 of the 122 chairmen who were identified for the study, resulting in an overall response rate of 80.3%. The mean ± SD age of respondents at the time of survey was 54.9 ± 6.8 years (range 37 to 71 years). More than half of the respondents (57%) were between 50 and 59 years old (Table I).

Twenty percent of the respondents had held more than one position as chairman, and most (63%) were working in a public medical school setting. The majority of respondents (66%) reported that they were acting as a mentor for a junior faculty member. Sixty-three percent considered themselves to be recognized leaders in their field (Table I).

Sixty-two percent of the chairmen were subspecialty certified compared with only 4.8% of all physicians certified in obstetrics and gynecology. Of the 61 chairmen holding subspecialty certification, 43% were certified in maternal-fetal medicine, 26% in reproductive endocrinology, and 31% in gynecologic oncology (Table II). This distribution is similar to the distribution of all subspecialists certified in the three subspecialties.

Training characteristics of the respondents before appointment are presented in Table III. Fifty-one percent had completed a subspecialty fellowship, 58% had been division directors, and 58% had been externally appointed. The majority of respondents (49%) were 40 to 49 years old when appointed (mean ± SD 46.3 ± 6.8, range 34 to 68). Most (76%) had achieved the highest academic rank (professor) before appointment.

Sixty-one percent reported that they had a mentor during their training, and 42 (43%) reported that they had received support from nationally recognized leaders in obtaining their appointment (Table III). Support consisted of letters of recommendation (86%), nominations for chairmanships (64%), and appointments to committees and organizations (36%).

Several statistically significant associations were found between training characteristics and age at the time of survey (Fig. 1). Younger chairmen (<50 years) were more likely than older chairmen (≥50 years) to be subspecialty certified (86% vs 56%, $p = 0.01$); younger chairmen were more likely to have completed a subspecialty fellowship (76% vs 44%, $p = 0.009$) and were *less* likely to be serving as mentors for junior faculty (38% vs 75%, $p = 0.001$).

Age was also found to be significantly associated with area of subspecialty certification (Fig. 2). Younger chairmen were more likely to be certified in maternal-fetal medicine (61% vs 35%) and reproductive endocrinology (33% vs 23%), whereas older chairmen were

Table I. Characteristics of chairmen at time of survey

Characteristic	*Respondents**	
	No.	*%*
Age (yr)		
<40	2	2.0
40-49	19	19.4
50-59	56	57.1
60-69	20	20.4
≥70	1	1.0
No. of chairs held		
1	78	79.6
2	17	17.3
3	3	3.1
Type of medical school		
Public	62	63.3
Private	28	28.6
Both	7	7.1
Current deparmental budget (millions)		
<$1	7	7.1
$1-$2	9	9.2
$2-$4	22	22.4
$4-$6	18	18.4
$6-$8	16	16.3
$8-$10	8	8.2
>$10	13	13.3
Subspecialty certified		
No	37	37.8
Yes	61	62.2
Length of tenure (yr)		
≤2	24	24.5
3-9	36	36.7
≥10	38	38.8
Acting as mentor for junior faculty member		
No	32	32.7
Yes	65	66.3
Member of nationally recognized leadership network		
No	32	32.7
Yes	62	63.3
Examiner for ABOG		
No	32	32.7
Yes	65	66.3

ABOG, The American Board of Obstetrics and Gynecology, Inc.

*Percentages are based on 98 respondents. Note that percentages do not always sum to 100 because some respondents did not answer all questions.

Table II. Subspecialty certification*

Subspecialty	National		Chairmen in survey	
	No.	%	No.	%
Maternal-fetal medicine	618	43.4	26	42.6
Reproductive endocrinology	400	28.1	16	26.2
Gynecologic oncology	407	28.6	19	31.1
Total subspecialty certified†	1,425	4.8	61	62.2
Total certified	29,908		98	

*Based on December 1990 statistics of The American Board of Obstetrics and Gynecology, Inc.
†Percentages are based on total number certified.

Table III. Training characteristics of chairmen before appointment

Characteristic	Respondents*	
	No.	%
Completed subspecialty fellowship		
No	48	49.0
Yes	50	51.0
Division director		
No	41	41.8
Yes	57	58.2
Examiner for ABOG		
No	48	49.0
Yes	50	51.0
Type of appointment		
Internal	40	40.8
External	57	58.2
Both	1	1.0†
Age at appointment (yr)		
<35	2	2.0
35-39	17	17.3
40-49	48	49.0
50-59	28	28.6
≥60	3	3.1
Academic rank		
Assistant	3	3.1
Associate	21	21.4
Professor	74	75.5
Had a mentor		
No	38	38.8
Yes	60	61.2
Received support in obtaining appointment from nationally recognized leaders		
No	48	49.0
Yes	42	42.9

*Percentages are based on 98 respondents. Note that percentages do not always sum to 100 because some respondents did not answer all questions.
†Held two chairs.

more likely to be certified in gynecologic oncology (42% vs 6%, $p = 0.02$).

In ranking the importance of leadership qualities and skills leading to appointment, respondents felt that interpersonal skills and knowledge of specialty were most important (Table IV). However, in the role of advising others, respondents ranked administrative skills as the most important skill requiring emphasis, followed by interpersonal skills. Two statistically significant differences were found between the mean rank of qualities leading to appointment and qualities stressed when advising others. Teaching skills were ranked significantly lower in importance when advising others ($p = 0.01$), and financial management skills were ranked significantly higher ($p = 0.001$).

Respondents were also asked to identify qualities that were lacking in themselves but that they thought were needed in the role of chairman. Financial management skills, political awareness, administrative skills, and research experience were identified as the four most lacking skills (67%, 39%, 32%, and 18%, respectively) (Table V).

The importance of support and experiences encouraged by mentors is presented in Table VI. For those chairman who had a mentor before appointment, providing administrative duties and gaining introductions to specialty leaders were ranked as the two most important areas stressed by their mentors. In serving as mentors to others, these same chairmen reported the two most important areas to be assumption of administrative duties and encouragement of research productivity.

Mentorship was found to be significantly associated with several training characteristics (Fig. 3). Those chairmen who had a mentor before their appointment were more likely to control a larger departmental budget (>$4 million) ($p = 0.002$), to have completed a subspecialty fellowship ($p = 0.008$), to have received support in obtaining their appointment from recognized leaders in the field ($p = 0.03$), and to have been an examiner for the American Board of Obstetrics and Gynecology, Inc., before their appointment ($p = 0.03$).

Several associations were found between size of department budget and training characteristics (Fig. 4). Those chairmen who were in charge of budgets in excess of $4 million were more likely to be subspecialty certified ($p = 0.03$), to have completed a subspecialty fellowship ($p = 0.001$), and to have been division director before appointment ($p = 0.03$).

Importance of leadership qualities and skills by

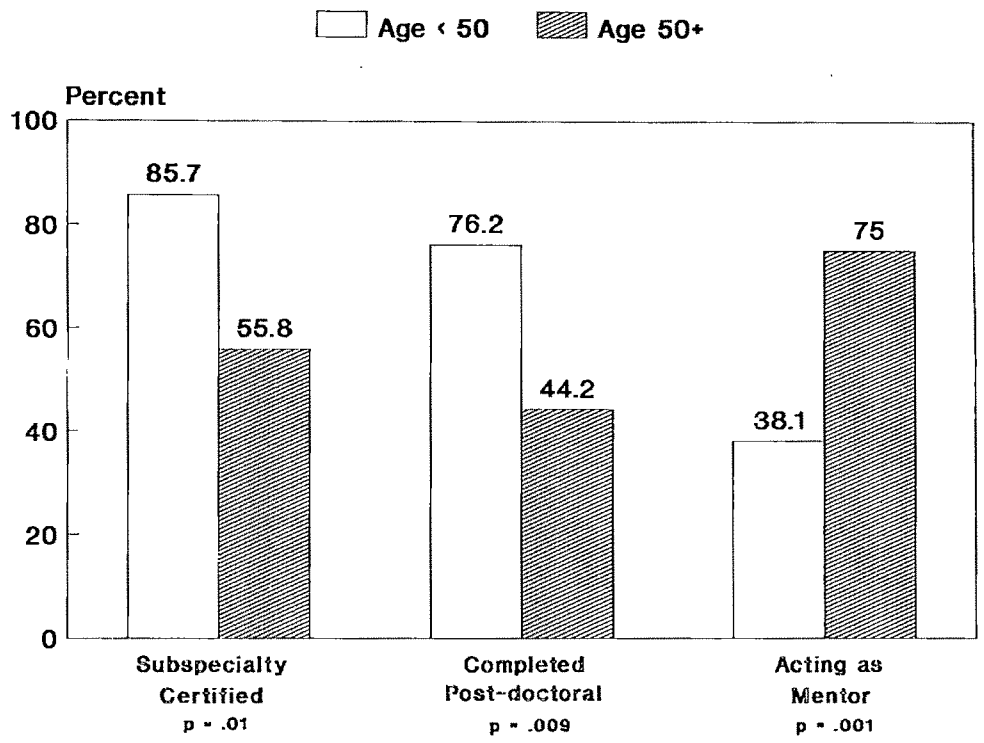


Fig. 1. Characteristics of chairmen by age at time of survey. p Value from χ^2 test for difference in proportions.

length of tenure in the chair is presented in Table VII. Chairmen holding office ≤2 years believed that respect within the specialty was the most important quality leading to their appointment. Those holding office 3 to 9 years thought that knowledge of the specialty was the most important quality, whereas those holding office ≥10 years ranked interpersonal skills as the most important of skills leading to appointment. In helping to develop skills in others, those holding office >10 years ranked knowledge of the specialty as the most important quality. However, those serving for <10 years thought that administrative skills were most important. The skill found to be most frequently lacking among all chairmen, regardless of length of tenure, was skill in financial management. In ranking skills that were encouraged by their mentors, all chairmen ranked administrative skills as most important.

Before appointment, all 98 (100%) of the respondents belonged to the American College of Obstetricians and Gynecologists. After appointment to chairman, 25 respondents (26%) became members of The American Gynecological and Obstetrical Society; 16 respondents become members of the Association of Professors of Gynecology and Obstetrics. The distribution of total membership within each society is presented in Table VIII.

The dean of the medical school was the most sought after individual for advice pertaining to the respondent's chairmanship (35% ranked as No. 1) (Table IX). Other persons often asked for advice were chairmen of other departments or other senior faculty members in obstetrics and gynecology. Very few respondents sought advice from outside of the medical school.

Only 49 (50%) of the respondents reported that they had been provided with opportunities by their medical school to learn management principles after their appointment. Of these 49, 59% had taken management short courses, 47% had attended Association of American Medical Colleges leadership seminars, and 39% had attended teaching workshops. (Totals exceed 100% because of multiple responses.)

Eighty-seven percent recommended that newly appointed chairs take a short course (2 to 16 weeks) in administration and financial management. However, only 28% of those recommending a short course had actually taken such a course. The majority of chairmen (83%) felt that national organizations in obstetrics and gynecology should develop leadership workshops. Most (77%) responded that the Associations of Professors of Gynecology and Obstetrics would best serve this need.

The majority of respondents (65%) believe that the traditional academic search committee is an effective method for selecting academic leaders. However, several criticisms of the traditional search committee were addressed by the respondents. Most criticisms were directed at the following issues: (1) There is a lack of

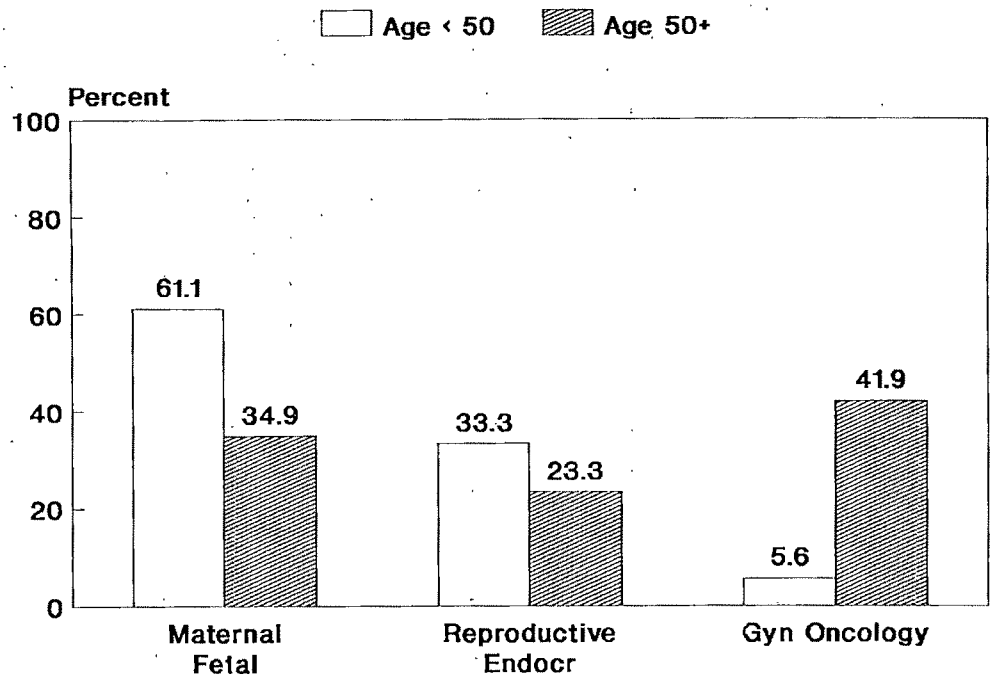


Fig. 2. Area of subspecialty certification by age at time of survey. $p = 0.02$ from χ^2 test for difference in proportions.

Table IV. Ranked importance of leadership qualities and skills

*Rank**	*Qualities leading to appointment*	*Qualities stressed in mentoring others*
1	Interpersonal skills	Administrative skills
2	Knowledge of specialty	Interpersonal skills
3	Administrative skills	Knowledge of specialty
4	Respect in specialty	Vision
5	Vision	Respect in specialty
6	Teaching skills	Financial management skills†
7	Communications skills	Research experience
8	Research experience	Communications skills
9	Political awareness	Teaching skills‡
10	Financial management skills	Political awareness
11	Social skills	Social skills

*Ranking: *1*, Most important: *11*, least important. Ranks are based on mean rank from all respondents.
†Ranked significantly higher, $p = 0.001$.
‡Ranked significantly lower, $p = 0.01$.

representation from members of the specialty of obstetrics and gynecology; (2) the search process is too slow; (3) the search committee may not be aware of the special needs of a department of obstetrics and gynecology; (4) there may be conflicting interests of search committee members.

Comment

"Successful corporations don't wait for leaders to come along. They actively seek out people with leadership potential and expose them to career experiences designed to develop that potential. Corporations that do a better-than-average job of developing leaders put an emphasis on creating challenging opportunities for relatively young employees."[16]

Leaders in academic medicine emerge from those who distinguish themselves in research and—to some extent—in teaching and clinical skills.[17] However, unlike in corporations, where careers begin in a person's mid-20s, in academic medicine one's career does not begin until age 30 or beyond. Therefore the preparation of future leaders in academic medicine begins relatively late.

In our opinion the most important finding from this study was the role of the mentor in developing the qualities and skills needed to become chairman of a department of obstetrics and gynecology. When Ulysses, King of Ithaca, picked his friend Mentor to advise his son Telemachus, the role of the mentor was established.[18] According to Levinson,[19] the mentor is teacher,

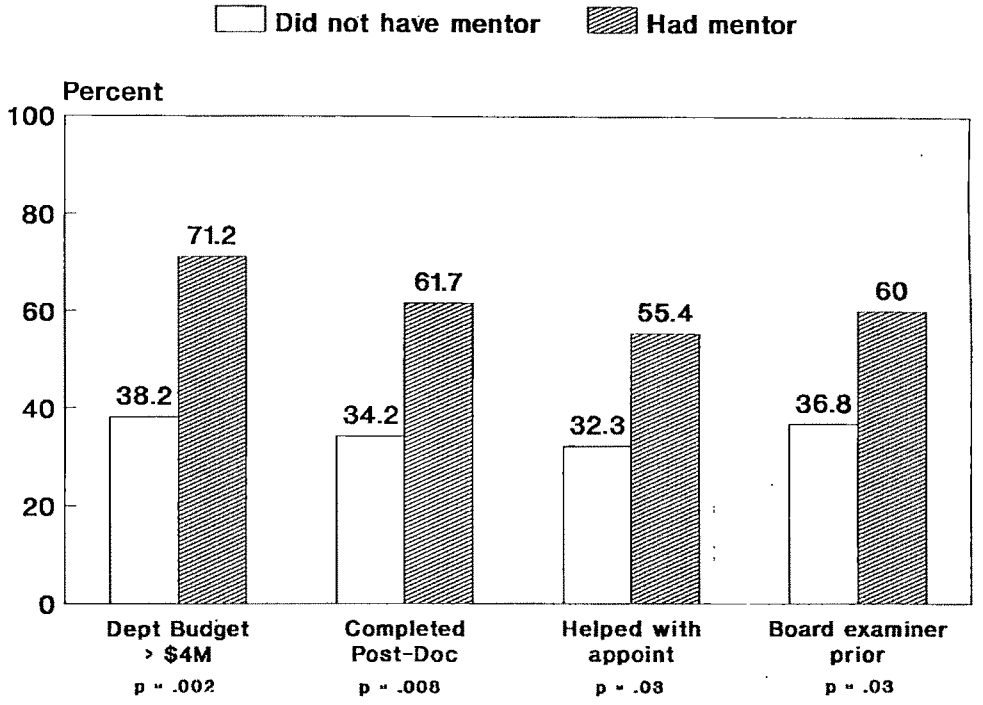


Fig. 3. Chairmen characteristics by mentorship. p Value from χ^2 test for difference in proportions.

counselor, sponsor, developer of skills, guide, and exemplar.

Departmental chairmen who had mentors before appointment were more likely to command department budgets in excess of $4 million, were more likely to have completed a subspecialty fellowship, and were more likely to have received support from recognized leaders in the specialty in obtaining their appointment.

The most important skill that chairmen reported their mentors had stressed to them was assumption of administrative duties, followed in importance by introductions to leaders in the specialty. However, when *serving* as mentors, department chairmen thought that encouragement of research productivity was the second most important experience to enhance in their protégés (protégées), followed by introductions to leaders in the specialty.

Department chairmen who had mentors were also more likely to be examiners for The American Board of Obstetrics and Gynecology, Inc., before their appointment as chairman. This finding may reflect the influence of the mentor and the support of leaders in the specialty in promoting emerging academic leaders to become board examiners.

The importance of mentors in developing future leaders of corporate enterprises is stressed in the management literature[20] and is now becoming stressed more frequently in the development of academic leaders.[21, 22] The classic mentor-protégé relationship in academic medicine is the relationship between William Osler and Harvey Cushing.[23] Academic leaders who have an enduring reputation are often remembered for their development of other academic leaders. Some medical schools and individual departments are noted for the production of academic leaders to fill leadership positions in other institutions.[24]

In this study 66% of chairmen reported that they were serving as mentors for future academic leaders. However, only 38% of chairmen under age 50 were serving as mentors, while 75% of those chairmen older than age 50 were serving as mentors for future aca-

Table V. Qualities identified as lacking on becoming chairmen

Quality	*Respondents**	
	No.	*%*
Financial management skills	66	67.3
Political awareness	38	38.8
Administrative skills	31	31.6
Research experience	18	18.4
Vision	7	7.1
Communications skills	6	6.1
Social skills	6	6.1
Interpersonal skills	5	5.1
Teaching skills	4	4.1
Knowledge of specialty	2	2.0
Fund-raising experience	1	1.0
Grant writing skills	1	1.0

*Percentages are based on 98 respondents. Multiple answers were allowed; therefore percentages do not sum to 100.

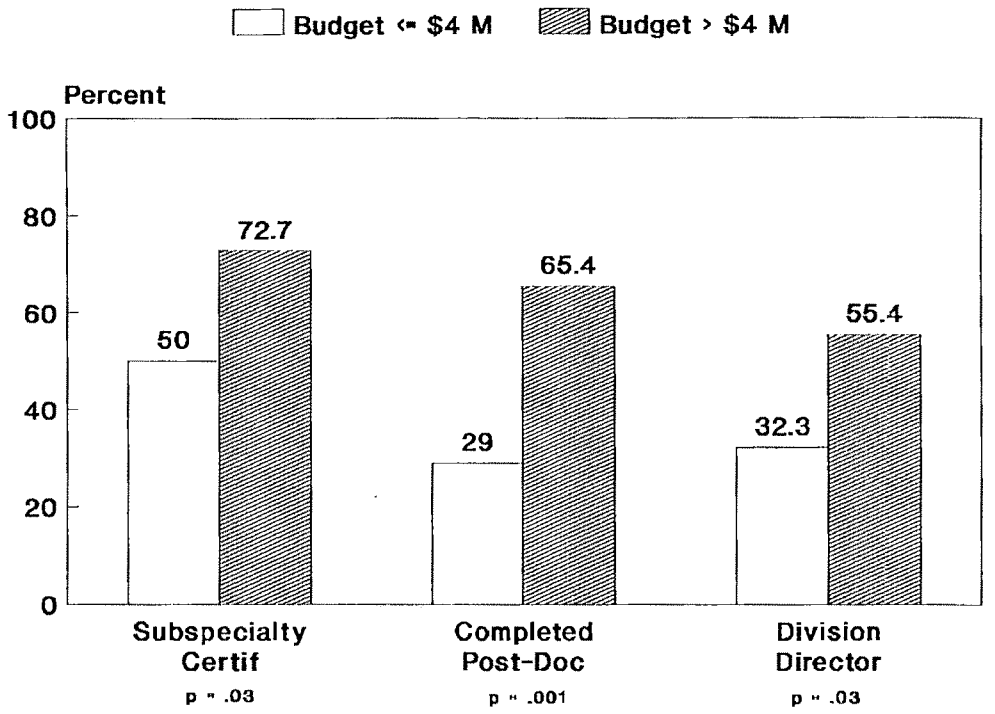


Fig. 4. Characteristics of chairmen by current departmental budget. p Value from χ^2 test for difference in proportions.

demic leaders. It is not surprising that younger chairmen are less likely to serve as mentors because they may need several years on the job as chairmen to establish credibility with faculty, to gain self-confidence, and to identify potential leaders. However, a newly appointed chairman should not wait too long to assume the mentor role.

We could not discern from this study when or how in a person's academic career the mentor-protégé (protégée) relationship was established. For the protégé (protégée) the relationship coulde begin as early as medical student days, during residency training, during the subspecialty fellowship, or on becoming a faculty member. We suspect that an early relationship fosters thoughtful career development. The mentor can serve as a role model in many academic areas such as teaching, basic and clinical research, and clinical practice. The mentor can open the door for the protégé (protégée) to meet leaders in the specialty and to participate in the activities of academic societies. The mentor can make the protégé (protégée) aware of career opportunities that will lead to career advancement.

Although it is the common belief that a mentor (or mentors) has a positive effect on an individual's career development, there are possible negative effects as well. If the mentor is not held in high esteem within the specialty, he or she may not be able to provide an entrée for the protégé (protégée). If the protégé (protégée) becomes a threat to the mentor, an adversarial relationship may develop, which may tarnish the mentor and/or the protégé (protégée). The mentor may provide poor career guidance with resultant negative consequences for the protégé (protégée).

We have no indication from this study as to how women chairmen and emerging women leaders in academic obstetrics and gynecology find mentors to guide career development. Kronik[20] emphasizes the importance of men serving as mentors for women faculty members until large numbers of women reach top academic administrative positions. He also advocates women serving as mentors for men.

Speroff[25] reported a decade ago on the tasks of chairmen of obstetrics and gynecology departments of medical schools and on the leadership qualities of chairmen. He emphasized the changing demands away from the chairman's role as teacher, clinician, and investigator and the increasing demands to provide financial resources. There have been increasing administrative demands placed on chairmen since Speroff's report. Of the chairmen responding to Speroff's questionnaire, 29% held subspecialty board certification, compared with 62% in the current study. Of the chairmen <50 years old in the current study, 86% held subspecialty board certification. This finding suggests that 10 years from now nearly all chairmen of departments of obstetrics and gynecology will hold subspecialty board certification. Moreover, younger chairmen were more likely to be certified in maternal-fetal medicine and less

Table VI. Importance of support and experiences provided by mentors

Activity	Rank*	
	Had mentor before appointment (n = 41)	*Acted as mentor to others (n = 48)*
Gave administrative duties	1	1
Introduced to specialty leaders	2	3
Nominated for society membership	3	4
Encouraged research productivity	4	2
Encouraged development of teaching skills	5	6
Nominated as candidate for chairs	6	7
Gave budget to manage	7	5

*Ranking: *1*, Most important: *7*, least important. Ranks are based on mean rank from number of respondents.

Table VII. Importance of leadership qualities and skills by length of tenure as chairman

	Length of tenure		
	≤2 yr	*3-9 yr*	*≥10 yr*
Qualities leading to appointment			
1	Respect	Knowledge	Interpersonal
2	Interpersonal	Interpersonal	Administrative
3	Vision	Respect	Knowledge
Qualities stressed in mentoring others			
1	Administrative	Administrative	Knowledge
2	Interpersonal	Interpersonal	Interpersonal
3	Vision	Vision	Administrative
Qualities lacking on becoming chair			
1	Financial	Financial	Financial
2	Administrative	Political	Political
3	Political	Administrative	Administrative
Skills encouraged by mentors			
1	Administrative	Administrative	Administrative
2	Introductions*	Research	Research
3	Nominations†	Introductions	Introductions

*Introductions to leaders in the specialty.

†Nominations to obstetrics and gynecology societies.

likely to be certified in gynecologic oncology. The number of reproductive endocrinologists has increased slightly among the younger chairmen.

The continuing increase in subspecialists who become departmental chairmen stands in stark contrast to the proportion of all certified obstetrician-gynecologists who hold subspecialty certification (4.5%). We assume that subspecialists, as a result of a subspecialty fellowship, have formal training in basic and clinical research, which gives them an added dimension as investigators and as leaders of research teams. Moreover, subspecialty fellowship training may give added experiences in teaching resident physicians and medical students. Our study does not provide data that allow us to compare the research and teaching experiences of physicians who completed subspecialty fellowships and became chairman with those of physicians who did not complete a subspecialty fellowship.

Does this trend toward selecting as department chairmen those who have acquired subspecialty certification foreclose the chair for those who are not subspecialty certified? We hope not! Subspecialty fellowship training and subspecialty certification do not impart special leadership qualities and should not be prerequisite to the chair. Many outstanding department chairmen, present and past, have distinguished themselves in general obstetrics and gynecology as teachers, investigators, and national leaders in the specialty. Our specialty will continue to need the broad vision of the generalists to maintain the integrity of obstetrics and gynecology. Most who complete residency training in obstetrics and gynecology enter general practice of the specialty (95.5%); therefore a broad view of the specialty must be maintained in academic centers. This does not imply that subspecialists have too narrow a focus to lead a broad discipline but does mean that subspecialists who become departmental chairmen must broaden the scope of their linking as they move up the administrative ladder.

In the current study chairmen ranked interpersonal skills as the most important quality leading to their chair appointment. This was followed in importance by

Table VIII. Membership in professional organizations

Organization	Member before appointment		Became member since appointment		Total now members	
	No.	%	No.	%	No.	%
American College of Obstetricians and Gynecologists	98	100	—	—	98	100
Association of Professors of Gynecology and Obstetrics	71	72.4	16	16.3	87	88.8
Society for Gynecologic Investigation	41	41.8	4	4.1	45	45.9
American Fertility Society	34	34.7	5	5.1	39	39.8
Central Association of Obstetricians and Gynecologists	30	30.6	10	10.2	40	40.8
Society of Perinatal Obstetricians	24	24.5	3	3.1	27	27.6
Society of Gynecologic Oncologists	20	20.4	2	2.0	22	22.4
American Gynecological and Obstetrical Society	20	20.4	25	25.5	45	45.9
South Atlantic Association of Obstetricians and Gynecologists	11	11.2	6	6.1	17	17.3
Pacific Coast Obstetrical and Gynecological Society	4	4.1	3	3.1	7	7.1

Table IX. Persons from whom chairmen seek advice

Person	Ranked as No. 1*	
	No.	%
Dean of medical school	34	34.7
Chairman of another department	27	27.6
Senior faculty in obstetrics and gynecology	26	26.5
Another chairman of obstetrics and gynecology	24	24.5
Former mentor	17	17.3
Former chairman of department	17	17.3
Spouse	14	14.3
Junior faculty in obstetrics and gynecology	6	6.1
Other	5	5.1
Nonphysician friend	4	4.1

*Percentages are based on 98 respondents. Percentages do not sum to 100 because many respondents ranked several persons as 1.

knowledge of the specialty, administrative skills, respect in the specialty, and vision. When advising others as mentors, chairmen ranked the same five qualities as most important; however, the order of importance was changed. Administrative skills were ranked first, followed by interpersonal skills, knowledge of specialty, vision, and respect in the specialty. The qualities selected as most important are consistent with those needed to achieve the leader's role in developing an understanding of fellow workers and the relationship of individual goals to departmental goals.

The qualities identified as lacking were financial management skills, political awareness, administrative skills, and research experience. Financial management skills, political awareness, and administrative skills can be developed more fully once a person has been selected as department chairman. These skills are not essential for selection to a leadership role but are essential to success in the leadership role.[26] When asked how these deficiencies were overcome, most chairmen responded with comments such as "on-the-job training," "course work," "hard work," and "still working on it." It is clear that chairmen are striving to attain the management skills necessary to lead their departments successfully. Those who listed lack of research experience as a deficiency are unlikely to overcome this deficiency once they become chairmen.

With the increasing managerial role of department chairmen to do things other than teach, provide clinical care, and perform research, medical schools have the responsibility to provide managerial education and support for newly appointed department chairmen. Speroff wrote: "Chairs need the assistance of qualified, able business people. It is unrealistic to expect experienced performance from untrained people. Surely it is the responsibility of the medical school and hospital to see that chairs are properly instructed and assisted, and never is this need greater than with a brand-new chair."[27] Only 50% of the respondents reported that they had been provided with opportunities by their medical schools to learn management principles. Those who had opportunities to study management principles took short courses in management or attended leadership workshops sponsored by the Association of American Medical Colleges.

Eighty-three percent of the respondents answered

that national organizations of obstetricians and gynecologists should develop leadership workshops and seminars for department chairmen and potential leaders in obstetrics and gynecology. Seventy-six percent of those who advocated leadership workshops for newly appointed department chairmen reported that the Association of Professors of Gynecology and Obstetrics would best be able to serve this need. Indeed, that organization has taken the initiative to survey needs of departmental chairmen and to develop workshops for acquiring managerial skills.

From this study we see the trend moving in the direction that chairmen of departments of obstetrics and gynecology of the future will more likely have completed a subspecialty fellowship and will more likely be subspecialty board certified than those appointed a decade earlier. Moreover, with the increasing managerial responsibilities of department chairmen, it is likely that future department chairmen will have gained some administrative experience before assuming the chair. Chairmen must recognize the importance of serving as mentors for future academic leaders. Medical school deans must recognize the necessity for providing newly appointed department chairmen with opportunities for management courses and administrative support.

We are concerned that research experience and teaching skills received relatively low ranking as leadership qualities and skills that led to appointment as department chairman. Emphasis on teaching skills was not being stressed in others who are being groomed for leadership positions, but the importance of research experience was acknowledged and is being emphasized. In our opinion the specialty will be advanced by the creation of new knowledge and by the ability to impart the knowledge of the discipline to medical students and young physicians who choose obstetrics and gynecology as a career specialty. As the onerous burden of administration increases, department chairmen cannot lose sight of the academic core of obstetrics and gynecology.

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"Chairman" is a troublesome term because of the potentially sexist connotations. Houle[10] reported that three fourths of two panels of women community leaders, diversified by age and race, who were consulted in 1985 and 1987 revealed a preference for the term "chairman" as the term they would most like to have applied to themselves. An equal proportion expressed strong aversion to "chairperson." Consequently, chairman is used throughout this manuscript without prejudice to refer to women and men who occupy the office of chair of a department of obstetrics and gynecology. (Adapted from Green et al.[11])

Discussion

DR. PRESTON V. DILTS, Ann Arbor, Michigan (Fellow). This is an area in which I have had some interest. I distinctly remember receiving and completing the survey and wondering about the results. It is especially interesting to be singled out in that I am one of the three who has been a chairman in three departments. I will not identify the others although I suspect they are easily recognizable.

Dr. Bates has analyzed our discipline well. There really is a wealth of data provided. Most of the data are not surprising.

The most consistent deficits in training for aspiring or new chairmen are in financial and management skills. Many chairmen and Dr. Bates recommend that such training be provided. Given the time required to attend courses sponsored by the Association of American Medical Colleges or others, perhaps Association of Professors of Gynecology and Obstetrics, The American Gynecological and Obstetrical Society, Inc., and/or the American College of Obstetricians and Gynecologists should develop programs designed for us. Association of Professors of Gynecology and Obstetrics is currently working on this.

The significant unimportant characteristic noted in the survey is teaching skill. I am puzzled because I had thought teaching to be the name of the game. I am further confused by having The American Board of Obstetrics and Gynecology, Inc., examiner status listed as highly important. Dr. Charles Hunter would be upset—one cannot examine unless one can teach.

Dr. Bates concludes that two factors seem to be most helpful in becoming a department chairman. One is being an examiner for the oral examinations of The American Board of Obstetrics and Gynecology, Inc., and the second is having a mentor. I believe these two are interrelated in that examiners are generally nominated to the board, usually by a mentor. Therefore Dr. Bates has one conclusion that states that the best way to become a department chairman is to have a good mentor or mentors. I think Dr. Bates is correct. I can name several people who have been very helpful in my own career and I answered the question yes, that I had mentors who had helped me.

The one thing Dr. Bates has not looked at, which I find a little distressing, is the issue of gender and diversity. I was able to locate my copy of the survey and find it did not ask for sex, race, or ethnic background. I suspect it would be difficult to identify ethnic background by names or return addresses. It might be possible to identify gender and race. Having said that, I am not sure what purpose it would serve because most of us can readily identify that there are very few women serving as chairmen, as well as very few minorities.

Since I feel strongly that we should represent our discipline, we must have more chairmen who are women and/or minorities. Dr. Bates acknowledges that gender is a problem. My question, and also my problem, then is how do we provide adequate mentoring for these young people so that they can become chairmen?

Clearly, in the short run, there will not be sufficient numbers of women or minorities who can be mentors for young faculty. This means that The American Gynecological and Obstetrical Society, Inc., and others must seriously look to advancing the careers of women and minorities in obstetrics and gynecology. We must have the goal of fostering, developing, and mentoring for women and minorities so they may become leaders in the discipline in the twenty-first century.

I look forward to any comments Dr. Bates may have. I very much appreciate the opportunity to discuss this interesting article. I think his conclusions are correct and represent a major area for concentration for the membership of The American Gynecological and Obstetrical Society, Inc.

Dr. Frederick P. Zuspan, Columbus, Ohio. This is a very gutsy topic for a group such as this, and I commend you for that. One thing I did learn is that 34% of the chairmen in this country probably don't do things correctly because they use the dean as their ancillary guide.

In my experience that would be the last person I would use as a confidant. I've always thought that the chairmen were the hard-core fiber of a medical school because deans sort of come and go.

The other thing I wondered if you had included in your survey, or had considered, is that most of the people in this room have probably served on the "search-and-seize" committees, and it's been my impression that people sort of weigh the volume and the content of the curriculum vitae and they look very critically at the number of publications. In other words, what type of a scientist is the individual for the appointment? None of them really ask whether the characteristics identified are present. In other words, is the candidate a good human being? Does he/she have good communication skills? Does he know how to get along with other chairmen and is he a financial genius with a master's degree in business?

I guess the question is how can we be prepared to become chairmen? That's one of the issues. Most chairmen are probably as unprepared as I was when I became chairman, and the issue is how that can be altered. Perhaps a mentor might be a solution but probably not a good one because of the perpetuation of evil or the perpetuation of error. It depends on who the mentor is.

Dr. Ronald Gibbs, Denver, Colorado. I have two brief questions. The first is did you look at current or previous research support, especially peer review in the background of the chairs? Second, you listed the rank order in leadership skills as perceived by the chairs with interpersonal skills being number one. As each chair is different, I wonder whether you could give us the information on the number of respondents listing other leadership skills as their number one choice.

Dr. Raymond H. Kaufman, Houston, Texas. One point that was not addressed, which does have some impact on the selection of the chairman, is input from the deans of the medical schools as to what they consider to be the necessary qualities for a departmental chairman. I find that some deans suffer from what I call "the dean's fantasy." They have the idea that out there is the ideal person with great administrative skills, significant research expertise, and lots of funds to bring into the department. The deans are the ones who direct the search committee as to the type of individual they want, and it would be of some interest to have an idea of exactly what our deans are looking for.

Dr. Frederick Naftolin, New Haven, Connecticut. I think you've done us a tremendous service because you've focused something that has been kind of un-

resolved, as it were, looking at what we're becoming. There is an ascendancy of the fellowship in academic medicine, and the mandatory nature of the fellowship adds 50% to each person's clinical training. There is a decline of the role of research in the discipline. When I put these two together, that is, the apparent inclusion of a fellowship as a requirement for becoming a faculty leader and chairman and the decline of research, it seems to me that this puts the burden of the maintenance of research on the subspecialty boards.

If the subspecialty boards do not do this, then the decline of research is almost assured because the addition of the 2 years of clinical training and the tremendous emphasis on the fiscal and service side of medical school departments stand to reduce us to the craftsmen that we were known to be when obstetrics was the lower third of the class.

I think the message that Dr. Bates has given us is very important, and that message is that we must incorporate research into what we consider an adequate subspecialty training.

Dr. Robert Vinchman, Fort Worth, Texas. I'm a great believer in the mentor system. I think it has a tremendous influence. However, in the British system the mentor is very specifically allied to one person. If by any chance your mentor dies, you are finished. I hope that this will not be anything that our mentors here go through.

Dr. Kamran Moghissi, Detroit, Michigan. Can you tell us whether your data indicate what happens—what is the impact of chairmanship on the chairman and what happens to career development after the chair is taken?

Dr. Martin Stone, Stony Brook, New York. I guess the discussants proved you were right in your conclusions. This is also an indictment of deans, mentors, and our organization because I haven't heard the word teaching brought up again since you said nobody was interested in it.

Dr. Bates (Closing). Thank you all for your critical and affirming questions and responses. Dr. Dilts, I appreciate your critical review of the manuscript and the comments that you have made.

I would like to make a disclaimer. I do not believe that being an examiner for The American Board of Obstetrics and Gynecology, Inc., is important to being a chairman. It's just one of the things that fell out of the data, and I think it points to the role of the mentor in the relationship with the people who become examiners and the role of the mentor in helping promote careers.

I do think though that it is important for the examiner to be an expert clinician and an expert teacher, and those should be the criteria for that selection.

The issue of gender and diversity is an extremely important one. This was an anonymous survey, and because there are and were so few women and minorities serving as chairmen, it would be easy to identify them. When I submitted the survey, I pledged to each respondent that I would not know who was responding. I do not have that information.

However, I think the important question and perhaps the most important issue to be discussed today is the fact that we need to serve as mentors for women. Today, to my knowledge, there are only five women serving in chairs, and this has been a growing number. More than 20% of the practitioners in obstetrics and gynecology are women, and approximately 50% of the residents are women. I think it's just a matter of time before women will occupy the same proportionate number of leadership positions as residency positions.

Kronik, in a very powerful article written for academicians in comprehensive universities, stated that men must mentor women now. We cannot wait for women to reach leadership roles so that younger women can have mentors, but men in positions of responsibility must begin developing women for leadership positions. I would like to emphasize that.

Dr. Zuspan, I agree with you on the folly of going to the dean as the most frequent source of advice, although I think the dean is in a position to have an overview of the entire medical school and probably is a good source of information and advice in looking at the total picture of the clinical departments, the basic science departments, and where resources can be allocated. I'm not sure deans always understand the unique needs of departments.

You asked the question, how can a person be prepared to become a department chairman except for a mentor? I think the mentor is probably the best way to get a start, but organizations such as this one and others must recognize the need to develop leadership skills in individuals so that they're not overwhelmed when they assume a chair. The Association of Professors of Gynecology and Obstetrics is now working to develop short courses for young physicians who will be moving into chairs and chairmen who are already in the position.

Dr. Naftolin raised the question about the importance of the fellowship and the fellowship programs and the board ensuring that people completing fellowships have adequate research experience. The burden of research is falling on the fellowship programs.

Dr. Gibbs asked if we inquired how much research support a chairman had before assuming the chair and while serving in the chair. I do not have those data. He also asked a question about what percentage of department chairmen ranked interpersonal skills as number one. It was >60%, and about 35% ranked all the others as most important.

Interestingly, we did find some stratification. The chairmen who had served from 3 to 9 years and >10 years were more likely to rank interpersonal skills as number one, and those who had served ≤2 years tended to rank knowledge of the specialty and respect within the specialty as number one.

Dr. Vinchman made the point that under the British system, if a person loses a mentor, he is doomed. I hope that's not the case in our system. I don't think there is

a single mentor in a person's career, but there's an evolution of mentors as people move along the line.

Dr. Moghissi asked, what is the impact of being a chairman on the chairman's career? I don't know the answer to that question, but I was impressed with the length of tenure of the respondents. More than 38 chairmen had served ≥10 years in the position. The mean age at appointment was 46 years. So, if you assume that chairmen will serve until about age 63, that's a long term for success. By comparison, the tenure of a dean is about 3.2 years today. So the chairman has much greater staying power in the position. I think that the chairman tends to develop his own skills as a chairman.

Finally, Dr. Stone, thank you for your comments. I agree we must focus on teaching. After all, we are medical schools.

Appendix

A survey of leadership qualities of chairmen of United States medical school departments of obstetrics and gynecology

1. Your age ____
2. Your age at time of appointment to first chair ____
3. No. of chairs held ____
4. Type of medical school
   Public ____ Private ____
5. Approximate current departmental budget from all sources
   <$1 Million ____
   $1-≤2 Million ____
   >$2-≤4 Million ____
   >$4-≤6 Million ____
   >$6-≤8 Million ____
   >$8-≤10 Million ____
   >$10 Million ____
6. Do you hold subspecialty certification from The American Board of Obstetrics and Gynecology, Inc.?
   Yes ____ No ____
   If yes, which subspecialty?
   Maternal-Fetal Medicine ____
   Reproductive Endocrinology ____
   Gynecologic Oncology ____
7. Do you have other subspecialty interests (urogynecology, genetics, infectious diseases, etc.)?
   Yes ____ No ____
8. Did you complete a postdoctoral fellowship?
   Yes ____ No ____
9. What was your academic rank of the time of your appointment to department chairman?
   Assistant Professor ____
   Associate Professor ____
   Professor ____
10. Were you a division director before becoming department chairman?
    Yes ____ No ____
11. Did you practice obstetrics and gynecology or general medicine in a nonuniversity or military setting before becoming chairman?
    Yes ____ No ____
    Obstetrics and gynecology ____ General medicine ____ How many years? ____
12. Was your appointment to department chairman internal (you became chairman of the department in which you were a faculty member) or external (you were recruited as chairman to another medical school)?
    Internal ____ External ____
13. Which qualities, skills, and experiences did you have before becoming chairman that led to your appointment as chairman? Please rank in order of importance and give an indication of the relative importance (essential, important, or less important).
    Rank
    ____ Vision
    ____ Knowledge of specialty
    ____ Respect within specialty
    ____ Teaching skills
    ____ Research experience
    ____ Communication skills (language fluency)
    ____ Interpersonal skills
    ____ Political awareness
    ____ Social skills
    ____ Administrative skills
    ____ Budget and financial management skills
    ____ Other (specify)
14. Did you have a mentor before becoming department chairman?
    Yes ____ No ____

continued

Appendix cont'd

15. How did your mentor help you develop skills to become a department chairman? Rank in order of importance.
    ____ Encouraged research productivity
    ____ Encouraged development of teaching skills
    ____ Introduced me to leaders in the specialty
    ____ Nominated me for membership in societies
    ____ Nominated me as a candidate for chairs
    ____ Gave me administrative responsibilities
    ____ Gave me a budget to manage
    ____ Other
16. Did the national leadership network in obstetrics and gynecology participate in your appointment as chairman?
    Yes ____ No ____
    If yes, how?
    ____ Nominated me as a candidate for chairmanships
    ____ Wrote letters of recommendation and support
    ____ Appointed me to committees of organizations
    ____ Other
17. To which of the following organizations did you belong before becoming department chairman?
    ____ American College of Obstetricians and Gynecologists
    ____ South Atlantic Association of Obstetricians and Gynecologists
    ____ Central Association of Obstetricians and Gynecologists
    ____ Pacific Coast Obstetrical and Gynecological Society
    ____ Society for Gynecologic Investigation
    ____ Association of Professors of Gynecology and Obstetrics
    ____ American Fertility Society
    ____ Society of Perinatal Obstetricians
    ____ Society of Gynecologic Oncologists
    ____ American Gynecological and Obstetrical Society
    ____ Others you consider important (specify) ____
18. Before becoming department chairman, were you an examiner for The American Board of Obstetrics and Gynecology, Inc.?
    Yes ____ No ____
19. On becoming dependent chairman, which of the following qualities, skills, and experiences did you find lacking in yourself that were important for the job as chairman?
    ____ Vision
    ____ Knowledge of the specialty
    ____ Teaching skills
    ____ Research experience
    ____ Communication skills (language fluency)
    ____ Interpersonal skills
    ____ Political awareness
    ____ Social skills
    ____ Administrative skills
    ____ Budget and financial management skills
    ____ Other (specify)
20. How did you overcome these deficiencies? Describe briefly.
21. Do you recommend that a newly appointed department chairman have a short course (2 to 16 weeks) in administration and financial management?
    Yes ____ No ____
    If yes, did you take such a course?
    Yes ____ No ____
22. As department chairman, from whom do you seek advice? Rank in order of importance.
    ____ Former mentor
    ____ Former chairman of your department
    ____ Dean
    ____ Chairman of another department in same medical school
    ____ Another chairman of obstetrics and gynecology
    ____ Senior faculty member in obstetrics and gynecology
    ____ Junior faculty member in obstetrics and gynecology
    ____ Spouse
    ____ Nonphysician friend
    ____ Other
23. Since becoming department chairman, have you become more a part of the national leadership network in obstetrics and gynecology?
    Yes ____ No ____
24. To which of the following organizations have you become a member since becoming department chairman?
    ____ American College of Obstetricians and Gynecologists
    ____ South Atlantic Association of Obstetricians and Gynecologists
    ____ Central Association of Obstetricians and Gynecologists
    ____ Pacific Coast Obstetrical and Gynecological Society
    ____ Society for Gynecologic Investigation
    ____ Association of Professors of Gynecology and Obstetrics
    ____ American Fertility Society

continued

Appendix cont'd

___ Society of Perinatal Obstetricians
___ Society of Gynecologic Oncologists
___ American Gynecological and Obstetrical Society
___ Others you consider important (specify) ___

25. Since becoming department chairman, have you become an examiner for The American Board of Obstetrics and Gynecology, Inc.?
Yes ___ No ___

26. In advising a young faculty member to develop leadership qualities, skills, and experiences, which of the following would you emphasize? (Please rank and give order of relative importance: essential, important, or not important.)
Rank
___ Vision
___ Knowledge of specialty
___ Respect within specialty
___ Teaching skills
___ Research experience
___ Communication skills (language fluency)
___ Interpersonal skills
___ Political awareness
___ Social skills
___ Administrative skills
___ Budget and financial management skills
___ Other (specify)

27. Have you become a mentor for a potential leader in obstetrics and gynecology since becoming department chairman?
Yes ___ No ___
If yes, do you (rank in order of importance):
___ Encourage research productivity?
___ Encourage development of teaching skills?
___ Introduce to leaders of specialty?
___ Nominate protegé for membership in societies?
___ Nominate protegé as a candidate for chairs?
___ Give protegé administrative responsibilities?
___ Give protegé budget management responsibilities?
___ Other (specify)

28. Are you grooming a person to become your successor?
Yes ___ No ___
Do you think that person is likely to become a department chairman?
Yes ___ No ___

29. After becoming department chairman, did your medical school provide opportunities to develop leadership skills?
Yes ___ No ___
If yes, how?
___ Association of American Medical Colleges leadership seminars
___ Teaching workshops
___ Management short courses
___ Other (specify)

30. In your opinion, is the traditional academic search committee an effective method for selecting academic department chairmen?
Yes ___ No ___
Briefly explain answer.

Does your school use a search firm to acquire leaders?
Yes ___ No ___

31. Should national organizations of obstetricians and gynecologists develop leadership workshops and seminars for department chairmen and potential future leaders?
Yes ___ No ___
If yes, which of the following organizations could best serve this need?
___ American College of Obstetricians and Gynecologists
___ Association of Professors of Gynecology and Obstetrics
___ American Gynecological and Obstetrical Society
___ Other (specify)

Human ovarian 17-ketosteroid oxidoreductase: Unique characteristics of the granulosa-luteal cell and stromal enzyme

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OBJECTIVES: We attempted to test the hypothesis that distinct forms of the 17-ketosteroid oxidoreductase exist in the human ovary and to compare its activity in stroma obtained from normally cycling women and from hyperandrogenic women.
STUDY DESIGN: Human ovarian granulosa-luteal cell and stromal 17-ketosteroid oxidoreductase were examined in cell incubations and subcellular homogenates.
RESULTS: In subcellular homogenates of granulosa-luteal cells 17-ketosteroid oxidoreductase activity was greater in the cytosol fraction than in the membrane fraction. In contrast, in homogenates of both ovarian stroma and Leydig cells its activity was greater in the membrane fraction than in the cytosol fraction. At the substrate concentrations used estrone was a better substrate than androstenedione for the granulosa-luteal cell 17-ketosteroid oxidoreductase. In contrast, androstenedione was a better substrate than estrone for that in ovarian stromal and Leydig cell membranes. In incubations of ovarian stroma from hyperandrogenic women, significantly more testosterone accumulated in the medium per milligram of tissue than in the medium of incubations of ovarian stroma from normally cycling women (142 ± 48 vs 7.9 ± 7.5 pg testosterone per milligram of tissue per 48 hours, mean ± SD, $p < 0.05$). The ratio of testosterone to androstenedione was significantly higher in the medium of incubations of ovarian stroma from hyperandrogenic women than in that from normally cycling women (0.61 vs 0.25, mean, $p < 0.05$). The ratio of serum testosterone to androstenedione was significantly greater in hyperandrogenic women than in normally cycling control women (0.31 ± 0.11 vs 0.20 ± 0.03, mean ± SD, $p < 0.05$).
CONCLUSION: The localization (cytosol fraction) and substrate specificity (estrone) of the granulosa-luteal cell 17-ketosteroid oxidoreductase enzyme resembles that seen in human placenta. The localization (membrane fraction) and substrate specificity (androstenedione) of the ovarian stromal 17-ketosteroid oxidoreductase enzyme resembles that seen in Leydig cells. It may be one enzyme that exists in multiple forms or it may be two (or more) enzymes. In some hyperandrogenic women the ovarian stromal 17-ketosteroid oxidoreductase may be more active than in normally cycling women, contributing to an abnormally increased testosterone production rate. (Am J Obstet Gynecol 1992;166:1117-26.)

Key words: 17-ketosteroid oxidoreductase, granulosa-luteal cell, stromal enzyme

The 17-ketosteroid oxidoreductase enzyme converts the weak androgen androstenedione to the potent androgen testosterone and converts the weak estrogen estrone to the potent estrogen estradiol. The characteristics of the human 17-ketosteroid oxidoreductase enzyme in granulosa-luteal cells, ovarian stroma, and Leydig cells have not been examined in detail. To test the hypothesis that distinct ovarian forms of the 17-ketosteroid oxidoreductase may exist, the substrate specificity and subcellular localization of the enzyme was examined in human granulosa-luteal cells and ovarian stroma. These results were compared with those from Leydig cells. In addition, the activity of ovarian stromal 17-ketosteroid oxidoreductase was compared in stroma obtained from normally cycling women and from hyperandrogenic women.

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Material and methods

Granulosa-luteal cell incubations. Granulosa-luteal cells were obtained at the time of oocyte retrieval from women in a program of in vitro fertilization. The granulosa-luteal cells were prepared for incubation as previously described.[1] A 50% Percoll gradient was used to separate the granulosa cells from the red blood cells. Cells were incubated in replicates of four at a cell density of 10,000 cells per well with 1 ml Dulbecco's minimal essential medium without serum at 37° C with 5% carbon dioxide. The well diameter was 16 mm. At the end of a 24-hour incubation period the medium was collected and frozen at −20° C until the steroid assays were performed.

For steroid assay the medium was extracted twice

with 10 volumes of ether. The ether was evaporated under a stream of nitrogen, and the dried steroids were redissolved in isooctane and applied to Celite columns for chromatography. With isooctane and ethyl acetate as the mobile phase, progesterone, androstenedione, dihydrotestosterone, testosterone, estrone, and estradiol were sequentially eluted from the column.[2, 3] Steroids were then measured by radioimmunoassays with specific antibodies. The testosterone antibody had <2% cross reactivity for androstenedione. The androstenedione antibody had <4% cross reactivity for testosterone. The estradiol antibody had <2% cross reactivity for estrone. The estrone antibody had <5% cross reactivity for estradiol. The intraassay and interassay coefficients of variation were <10% and 15%, respectively, for all steroids assayed.

Granulosa-luteal cell fractionation. Granulosa-luteal cells were sonicated for three 10-second bursts at 4° C in 0.01 mol/L phosphate-buffered saline solution. The homogenate was centrifuged at 100,000*g* for 60 minutes. This supernatant was used as the cytosol. The pellet was suspended and homogenized in 0.01 mol/L phosphate-buffered saline solution with a Teflon pestle and glass vessel and used as the membrane fraction.

Measurement of 17-ketosteroid oxidoreductase activity in granulosa-luteal cell fractions. The assay for the 17-ketosteroid oxidoreductase used a reaction mixture containing 0.15 mol/L sodium chloride and 0.01 mol/L phosphate at pH 7.4 and 37° C, 2 mg/ml bovine serum albumin, 500 μmol/L reduced nicotinamide adenine dinucleotide phosphate, 50 mmol/L glucose-6-phosphate, 2 U of glucose-6-phosphate dehydrogenase, and appropriate concentrations of tritiated steroid and unlabeled steroid precursor (androstenedione or estrone) in a final volume of 0.4 ml.[2]

Three concentrations of substrate (estrone or androstenedione) were used: 25, 200, and 500 nmol/L. The protein concentration was 140 μg for the membrane fraction and 106 μg for the cytosol fraction per incubation. The incubation times were 3 and 12 minutes. Duplicate incubations were performed at each time point. To inhibit aromatase, 20 μmol/L aminoglutethimide was added to all incubations. The reaction was terminated by adding 0.4 ml methanol. The assay was linear with the time and protein content.[2] Steroids were extracted with ether and separated by Celite chromatography.

Incubations of human ovarian stroma. Ovarian tissue was obtained from seven hyperandrogenic and six nonhyperandrogenic women undergoing oophorectomy or ovarian cystectomy. All were in the follicular phase of the menstrual cycle. Stromal tissue was prepared from the ovarian specimens as previously described.[3] With a dissecting microscope the tunica albicans was stripped from the specimens, and all follicles >1.0 mm in diameter were dissected free of the stromal tissue. The stromal tissue was dissected into pieces <1.5 mm in maximum diameter. The minced stroma was washed twice in Dulbecco's minimal essential medium and randomly aliquoted to culture wells in a 24-well plate. Incubations were performed for 48 hours in quadruplicate in plastic culture wells with 1 ml Dulbecco's minimal essential medium with glutamine, penicillin, and streptomycin at 37° C in 5% carbon dioxide with air. At the termination of the 48-hour incubation the media were frozen at −20° C until steroid assays were performed.[2, 3] Differences in steroid accumulation in the incubation medium between hyperandrogenic and normal ovarian specimens were evaluated with both the *t* test and Wilcoxon rank sum test. Differences in the testosterone-to-androstenedione ratio were evaluated with the Wilcoxon rank sum test.

Measurement of 17-ketosteroid oxidoreductase activity in ovarian stroma fractions. Stroma was prepared from whole ovaries as noted above. The stroma was homogenized in a Brinkman system with 0.01 mol/L phosphate-buffered saline solution. The homogenate was sonicated with three 10-second pulses at 4° C. The sonicated homogenate was centrifuged for 10 minutes at 100*g* and the pellet was discarded. The supernatant was centrifuged at 100,000*g* for 60 minutes. This supernatant was used as the cytosol fraction. The pellet from the 100,000*g* centrifugation step was homogenized in 0.01 mol/L phosphate-buffered saline solution and used as the membrane fraction.

The assay for the 17-ketosteroid oxidoreductase was similar to that for the granulosa-luteal cells. Three concentrations of substrate were used: 200 nmol/L, 500 nmol/L, and 1 μmol/L.

Measurement of 17-ketosteroid oxidoreductase activity in Leydig cell fractions. Testes were obtained from orchiectomy specimens. Leydig cells were prepared as previously described.[2] The Leydig cells were disrupted by sonication with three 10-second pulses. The sonicate was centrifuged at 100,000*g* for 60 minutes. The supernatant was used as the cytosol fraction. The pellet was homogenized and used as the membrane fraction. The assay for the 17-ketosteroid oxidoreductase was similar to that for the granulosa-luteal cells. Two concentrations of substrate were used: 200 and 500 nmol/L.

Testosterone-to-androstenedione ratio in women. Serum testosterone, androstenedione, and progesterone were measured with ether extraction, Celite chromatography, and specific radioimmunoassays in 7 normally cycling women and 11 hyperandrogenic women.[2, 3] None of these women contributed ovarian specimens to the studies of ovarian stroma (see above). The normally cycling women were nonhirsute and had menstrual cycle lengths between 27 and 32 days. The

Table I. Distribution of 17-ketosteroid oxidoreductase in homogenates of granulosa-luteal cells estrone and androstenedione used as substrates

Substrate	*Product*	*Homogenate fraction*	*Substrate concentration*		
			25 nmol/L	*200 nmol/L*	*500 nmol/L*
Estrone	Estradiol	Cytosol	3.8	8.1	29.9
Androstenedione	Testosterone	Cytosol	1.1	3.9	10.8
Estrone	Estradiol	Membrane	—	2.1	5.5
Androstenedione	Testosterone	Membrane	—	1.8	3.5

Each value represents the mean of two incubations (picomoles per minute per milligram of protein).

11 hyperandrogenic women were oligomenorrheic and hirsute and had at least one elevated total testosterone concentration before entry into this study. Serum was obtained between 8 and 9 AM. All women were studied in the follicular phase of the menstrual cycle, as determined by a progesterone concentration <2 ng/ml. Non–sex hormone–binding globulin–bound testosterone was determined with an ammonium sulfate precipitation technique at 4° C.[4] Differences in the serum androstenedione and testosterone concentration between the hyperandrogenic and normally cycling women were evaluated with the *t* test. Differences in the ratio of testosterone to androstenedione and non–sex hormone–binding globulin–bound testosterone to androstenedione in the hyperandrogenic and normally cycling women were evaluated with the Wilcoxon rank sum test.

For all studies protein concentrations were determined by the method of Bradford.[5] Tritium-labeled steroids were obtained from Dupont (Boston) and purified by high-performance liquid chromatography on a C-18 micro-Bondapak column (Millipore, Bedford, Mass.) before use. Collagenase was obtained from Worthington Biochemical Co. (Freehold, N.J.). All other reagents were obtained from Sigma Chemical Co. (St. Louis).

Results

Granulosa-luteal cell incubations. In serum- and lipid-free medium, progesterone accumulated (22,000 ± 4500 pg/ml, mean ± SD) in the medium, but androstenedione, testosterone, dihydrotestosterone, estrone, and estradiol were not detected (<10 pg/ml). The addition of androstenedione to the medium resulted in a concentration-dependent increase in estradiol release into the medium over a range of androstenedione from 5 to 1000 nmol/L. Addition of 10,000 pg/ml androstenedione at time 0 to 10,000 granulosa cells resulted in the appearance of 506 ± 60 pg/ml estradiol, <40 pg/ml estrone, and <40 pg/ml testosterone in the medium at 24 hours of culture.

Addition of 1 μCi of tritiated progesterone or tritiated estradiol to 10,000 granulosa cells for 24 hours resulted in the conversion of <10% of the progesterone and <5% of the estradiol to other metabolites, as determined by high-performance liquid chromatography (C18-Bondapek, methanol-water liquid phase).

Localization and substrate specificity of 17-ketosteroid oxidoreductase activity in homogenates of granulosa-luteal cells. As noted in Table I, both the cytosol and the membrane fraction contained measurable enzyme activity with both estrone and androstenedione as substrates. At the substrate concentrations used, the majority of enzyme activity was localized to the cytosol fraction. In the cytosolic and membrane fraction, product formation was greater when estrone was used as the substrate than when androstenedione was used. At the substrate concentrations used, a maximal velocity was not achieved.

Incubations of human ovarian stroma. At time zero, stromal tissue contained <5 pg/mg tissue of androstenedione, testosterone, and progesterone and <1 pg/mg tissue of dihydrotestosterone, estrone, and estradiol. For all ovarian specimens, after 48 hours of incubation, testosterone and androstenedione concentrations in the medium were greater than that present in tissue at time 0 (Table II). The ovarian stroma from the hyperandrogenic women released significantly more testosterone into the medium over 48 hours of incubation than stroma from the nonhyperandrogenic women. The amount of androstenedione released into the medium was not statistically different between the two groups, in part because of the large variance observed in androstenedione accumulation among specimens. In the incubations of stroma from the nonhyperandrogenic women, 4.0 molecules of androstenedione accumulated in the media for each molecule of testosterone. In contrast, in the incubations of stroma from the hyperandrogenic women, 1.6 molecules of androstenedione accumulated in the media for each molecule of testosterone. The ratio of testosterone to androstenedione released into the medium was significantly higher in the incubations of stroma from hyperandrogenic women ($p < 0.05$).

Localization and substrate specificity of 17-ketosteroid oxidoreductase activity in homogenates of

Table II. Accumulation of androstenedione and testosterone in the medium of incubations of ovarian stroma from normally cycling women and hyperandrogenic women

	N	*Androstenedione (pg/mg tissue/48 hr)*	*Testosterone (pg/mg tissue/48 hr)*	*Testosterone-to-androstenedione ratio*
Control stroma	6	31.9 ± 11	7.9 ± 7.5	0.25
Hyperandrogenic stroma	7	232 ± 127	142 ± 48	0.61
t Test		NS	$p < 0.05$	—
Wilcoxon rank sum		NS	$p < 0.01$	$p < 0.05$

For each stromal sample, four incubations were performed to obtain a mean androstenedione and testosterone accumulation (mean ± SD).

NS, Not significant.

Table III. Distribution of 17-ketosteroid oxidoreductase in homogenates of ovarian stroma with estrone and androstenedione used as substrates

Substrate	*Product*	*Cell fraction*	*Substrate concentration*		
			200 nmol/L	*500 nmol/L*	*1 μmol/L*
Estrone	Estradiol	Cytosol	0.55	1.9	2.2
Androstenedione	Testosterone	Cytosol	0.54	1.3	3.3
Estrone	Estradiol	Membrane	1.8	8.1	5.3
Androstenedione	Testosterone	Membrane	3.3	9.1	15.8

Each value represents the mean of two incubations (picomoles per minute per milligram of protein).

ovarian stroma. Using stroma from a premenopausal ovary, 17-ketosteroid oxidoreductase activity was detected in both cytosolic and membrane fractions (Table III). At the substrate concentrations used, the majority of enzyme activity was in the membrane fraction when estrone or androstenedione was used as substrates. In the cytosolic fraction the amount of product formed was similar when estrone or androstenedione was used as substrate. In the membrane fraction, at substrate concentrations of 200 nmol/L and 1 μmol/L more product formation was observed when androstenedione was used as substrate than when estrone was used. In the data presented in Table III, 500 nmol/L estrone resulted in greater product formation than did 1 μmol/L estrone. This anomaly could be explained by experimental variability or a technical error. At the substrate concentrations used, a maximal velocity was not achieved. In an additional ovarian specimen from a premenopausal woman, when androstenedione was used as the substrate, 88% of the enzyme activity was in the membrane fraction and 12% of the activity was in the cytosol fraction. In an ovarian specimen from a menopausal woman, when androstenedione was used as the substrate, 100% of the enzyme activity was in the membrane fraction. No activity could be detected in the cytosolic fraction.

Localization and substrate specificity of 17-ketosteroid oxidoreductase activity in homogenates of Leydig cells. Enzyme activity was detected in the cytosol and membrane fractions of Leydig cells (Table IV). At the substrate concentrations used, the majority of activity was in the membrane fraction when estrone or androstenedione was used as substrate. In the membrane fraction at both substrate concentrations tested, more product was formed when androstenedione was used as substrate than when estrone was used.

Testosterone-to-androstenedione ratio in women. Serum androstenedione, total testosterone, and non–sex hormone–binding globulin–bound testosterone were significantly higher in the hyperandrogenic women than in the normally cycling control women (Table V). In comparing the two groups, androstenedione, testosterone, and non–sex hormone–binding globulin–bound testosterone were 104%, 217%, and 570% higher, respectively, in the hyperandrogenic women ($p < 0.05$) than in the control women. The testosterone-to-androstenedione ratio was 55% higher in the hyperandrogenic women than in the controls ($p < 0.05$). The non–sex hormone–binding globulin–bound testosterone-to-androstenedione ratio was 221% higher in the hyperandrogenic women than in the controls ($p < 0.05$).

Comment

Since the description by Ryan and Engel[6] of the interconversion of estrone and estradiol by human tissue slices, many investigators have proposed that human 17-ketosteroid oxidoreductase activity (also referred to as 17β-hydroxysteroid dehydrogenase, EC 1.1.1. 62) is composed of one enzyme that exists in multiple forms or is actually two or more enzymes.[7, 8] For example, Tseng et al.[7] reported that an antiserum

Table IV. Distribution of 17-ketosteroid oxidoreductase activity in Leydig cell homogenates with estrone and androstenedione used as substrates

Substrate	*Product*	*Cell fraction*	*Substrate concentration*	
			200 nmol/L	*500 nmol/L*
Estrone	Estradiol	Cytosol	0.2	0.5
Androstenedione	Testoste	Cytosol	0.5	1.2
Estrone	Estradiol	Membrane	9.4	31
Androstenedione	Testoste	Membrane	63	83

Each value represents the mean of two inc ations (picomoles per minute per milligram of protein).

Table V. Serum androstenedione, testost one, and non–sex hormone–binding globulin–bound testosterone concentration in normal cyc g women and hyperandrogenic women

	No.	*Age (yr)*	*Androstenedione (ng/ml)*	*T testo (ng*	*l one l)*	*Non–sex hormone–binding globulin bound testosterone (pg/ml)*	*Total testosterone-to-androstenedione ratio*	*Non–sex hormone–binding globulin bound testosterone-to-androstenedione ratio*
Normal cycling women	7	34	1.47 ± 0.35	0.29 ±	0.076	20 ± 9.1	0.020 ± 0.03	0.014 ± 0.007
Hyperandrogenic women	11	30	3.00 ± 1.5	0.92 ±	0.54	134 ± 123	0.31 ± 0.11	0.045 ± 0.034
t Test			$p < 0.05$	$p <$	05			
Wilcoxon rank sum						$p < 0.05$	$p < 0.05$	$p < 0.05$

Values are mean ± SD.

raised against human placental estradiol lehydroge-nase could inhibit placental 17-ketostero oxidored-uctase activity but could not inhibit end netrial 17-ketosteroid activity. They concluded that cental and endometrial forms of 17-ketosteroid ox oreductase were immunologically distinct proteins. omquist et al.[8] reported that human placenta may co in two distinct forms of 17-ketosteroid oxidoreducta One form recognized C_{18}-, C_{19}-, and C_{20}-steroids with omparable affinities, and this activity could be comple y inhibited by dihydrotestosterone. The second form f 17-keto-steroid oxidoreductase was highly specific estradiol, and this activity could only be partially hibited by dihydrotestosterone.

Evidence that the protein(s) responsibl for 17-ketosteroid oxidoreductase activity exists multiple physicochemical forms is provided by the bservation that 17-ketosteroid oxidoreductase activity s predominantly in the cytosolic fraction of some ti ues and is predominantly in the membrane fraction other tissues. Tissues and cells where the 17-keto eroid oxidoreductase activity is predominatly in t cytosolic fraction of homogenized tissue or cells inc de human placenta,[9] whole human ovary,[10] ovine ova ,[11] and rat granulosa cells.[2] In contrast, tissues and cel where the 17-ketosteroid oxidoreductase activity is predominantly in the membrane fraction of homogenized tissues and cells include the human endometrium,[7] rat testis,[12] canine testis,[13] porcine testis,[14] hamster testis,[15] and rat Leydig cells.[2] It is likely that the 17-ketosteroid oxidoreductase protein present in the cytosol of ovary and placenta is more hydrophilic than that present in the membrane fraction of testis and endometrium.

In the current study the majority of 17-ketosteroid oxidoreductase activity was present in the cytosolic fraction of granulosa-luteal cell homogenates regardless of whether estrone or androstenedione was used as the substrate. In homogenates of granulosa-luteal cells, 84% of the 17-ketosteroid oxidoreductase activity was in the cytosolic fraction at an estrone concentration of 500 nmol/L. In contrast, in homogenates of Leydig cells, the majority of the 17-ketosteroid oxidoreductase activity was in the membrane fraction whether estrone or androstenedione was used as the substrate. In homogenates of Leydig cells 99% of the 17-ketosteroid oxidoreductase activity was in the membrane fraction at androstenedione concentrations of 500 nmol/L. The ovarian stroma resembled the Leydig cell in that the majority of the 17-ketosteroid oxidoreductase activity was in the membrane fraction of the homogenate whether estrone or androstenedione was used as the substrate. In the human ovarian stroma 83% of the 17-

ketosteroid oxidoreductase activity was in the membrane fraction at the androstenedione concentration of 1 μmol/L. An important caveat is that the ovarian stroma is a complex tissue with blood vessels, lymphatics, nerves, small follicles containing granulosa cells and theca, and ovarian mesenchymal cells. The contribution of each of these cell types to the stromal 17-ketosteroid oxidoreductase activity is unknown. In one study of a postmenopausal ovary (no follicles), the 17-ketosteroid oxidoreductase activity was localized to the membrane fraction, suggesting that the small follicles present in the premenopausal ovarian stroma do not contain all of the stromal 17-ketosteroid oxidoreductase activity. At the substrate concentrations used, a maximal velocity was not achieved. Future studies will be required to determine the Michaelis-Menten constants and the maximal velocities for these enzymes.

Additional evidence that the protein(s) responsible for 17-ketosteroid oxidoreductase activity exists in multiple forms is provided by the observation that the rat granulosa cell and Leydig cell 17-ketosteroid oxidoreductase have different substrate specificities.[2] In the intact rat granulosa cell and in rat granulosa cell cytosol, estrone is a preferred substrate to androstenedione.[2] In contrast, in the intact rat Leydig cell and in rat Leydig cell microsomes, androstenedione is a preferred substrate to estrone.[2] In the current study in granulosa-luteal cell cytosol product formation was greater when estrone was used as a substrate than when androstenedione was used. In the ovarian stroma membrane preparation, at two substrate concentrations (200 nmol/L, 1 μmol/L), more product formation was observed when androstenedione was used as a substrate than when estrone was used. At a third substrate concentration, (500 nmol/L), product formation was similar with both substrates. Further information will be necessary to clarify the substrate specificities of the 17-ketosteroid oxidoreductase in human granulosa-luteal cells and ovarian stroma.

Recently, two genes for human 17-ketosteroid oxidoreductase have been cloned and sequenced and assigned to chromosome 17q12.[16-18] These two genes are in tandem and contain 89% homology in the overlapping exons and introns. The gene h17-β-hydroxysteroid dehydrogenase II encodes a protein of approximately 327 amino acids with a predicted molecular weight of 34,853.[17] The gene h17-β-hydroxysteroid dehydrogenase I may encode for a protein of approximately 214 amino acids, or it may be a pseudogene.[18] Of particular interest, the human placenta contains two messenger ribonucleic acid species for the 17-ketosteroid, a major species migrating at 1.3 kb and a minor species migrating at 2.2 kb.[17] The 1.3 kb species is also predominant in some human ovarian specimens.[18] The 2.2 kb species is predominat in endometrium, myometrium, uterus, breast, prostate, and fat tissue.[18]

Incubation of the granulosa-luteal cells in serum-free medium resulted in the accumulation of progesterone in the range of 2 pg per cell per 24 hours. Androstenedione, testosterone, estrone, and estradiol could not be detected in the medium. Addition of androstenedione to the medium resulted in the dose-dependent accumulation of estradiol, with minimal accumulation of estrone or testosterone. This finding of a low testosterone-to-androstenedione ratio but high estradiol-to-estrone ratio has also been reported by McNatty et al.[19] in human follicular fluid. In follicular fluid from healthy follicles, the steroid concentrations were androstenedione 930 ng/ml, testosterone 63 ng/ml, estrone 62 ng/ml, and estradiol 465 ng/ml.[19] This results in a testosterone-to-androstenedione ratio of 0.07 and an estradiol-to-estrone ratio of 7.5. The differences in the ratios of the 17-hydroxy to 17-keto forms of these two steroid pairs remains to be fully explained. The biologic effect is to allow for the accumulation of large amounts of the potent estrogen estradiol and minimal amounts of the potent androgen testosterone.

The incubations of ovarian stroma from normally cycling women resulted in the accumulation of testosterone and androstenedione in a ratio of 0.25 (testosterone to androstenedione). This is similar to the testosterone-to-androstenedione ratio observed by McNatty et al.[20,21] in incubations of human ovarian theca (0.04 to 0.13) and stroma (0.26 to 0.42) from normally cycling women. In the incubations of ovarian stroma from the hyperandrogenic women, significantly more testosterone accumulated and the testosterone-to-androstenedione ratio was significantly greater than that observed in the normally cycling women (0.61 vs 0.25, $p < 0.05$). These results suggest that one mechanism subserving the development of ovarian hyperandrogenism is an increase in stromal 17-ketosteroid oxidoreductase, resulting in an abnormally elevated testosterone-to-androstenedione ratio. McNatty et al.[21] studied the ovarian tissue of a woman with the syndrome of hyperandrogenism-insulin resistance and acanthosis nigricans.[22] This woman had a point mutation in both alleles of the insulin receptor gene, resulting in a nonfunctional insulin receptor because of the replacement of a valine for a phenylalanine at amino acid 382.[23] In incubations of ovarian stroma from this woman the testosterone-to-androstenedione ratio ranged from 3.94 to 20.5. This markedly abnormal ratio in the presence of a severely hyperinsulinemic state suggests that hyperinsulinemia may contribute to the stimulation of stromal 17-ketosteroid oxidoreductase activity.

If one mechanism subserving the development of ovarian hyperandrogenism is an increase in ovarian stromal 17-ketosteroid oxidoreductase, then women with hyperandrogenism may have an abnormal ratio of testosterone to androstenedione in the circulation.

The normally cycling women in this study had a serum testosterone-to-androstenedione ratio of 0.20. This is similar to that observed in normally cycling women by Aiman et al.[24] (0.19) and Chang et al.[25] (0.20). In this study the ratio of serum testosterone to androstenedione was significantly elevated in hyperandrogenic women compared with normally cycling controls (0.31 ± 0.11 vs 0.20 ± 0.03, $p < 0.05$). In future studies the measurement of the serum testosterone-to-androstenedione ratio in normal and hyperandrogenic women after adrenal suppression is planned to evaluate the ovarian and adrenal contributions to this finding.

In this study evidence has been presented that human ovarian stroma and granulosa-luteal cells may contain different forms of the 17-ketosteroid oxidoreductase enzyme. The ovarian stromal 17-ketosteroid oxidoreductase resembles the enzyme present in Leydig cells, and the granulosa-luteal cell 17-ketosteroid oxidoreductase resembles the enzyme present in the placenta. In some hyperandrogenic women, the ovarian stromal 17-ketosteroid oxidoreductase may be overexpressed, contributing to an abnormally increased testosterone production rate.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Howard C. Judd, (Fellow) Los Angeles, California. This study represents a comprehensive attempt to define the 17-ketosteroid oxidoreductase enzyme with both in vitro and in vivo methods. It falls into four basic studies. First, enzyme activity was localized to specific subcellular sites and was quantitated in granulosa-luteal cells obatined from patients undergoing ovarian hyperstimulation for in vitro fertilization. Second, similar experiments were performed with cells from the ovarian stroma obtained at surgery from normal and hyperandrogenized women. Third, the same experiments were performed on Leydig cells obtained after

orchiectomy. And, finally, enzyme activity was assessed indirectly by measurement of serum levels of androstenedione, testosterone, and non–sex hormone–binding globulin–bound testosterone obtained from normal and hyperandrogenized women.

In general, the methods used in this study were reasonably sophisticated, the statistical approach to the analyses of data was appropriate, the presentation of the results was clear, and the interpretation of the results was restrained and cautious. Thus Dr. Barbieri should be commended for his efforts. It should also be pointed out that Dr. Barbieri is a sophisticated reproductive endocrinologist and as such knows that studies of this nature are always open to criticism and concern. Thus his judicious and careful interpretation of his finding is to be complimented.

In critiquing Dr. Barbieri's study, I would like to address three issues. My task in being the official reviewer is to offer some criticisms. This critique should not be taken by anyone to indicate I disapprove of it, which I do not. The first is the possible error that could occur with metabolism of either substrate or product hormone. Dr. Barbieri quantitated enzymatic activity by exposing whole cells or cell fractions to substrate hormone and measuring product hormone by radioimmunoassay. As long as the metabolism of the substrate hormones androstenedione and estrone or the product hormones testosterone and estradiol was the same, then this type of comparison was reasonable. If it was not, then problems could exist. For example, 5α-reduction of androstenedione and testosterone is known to occur but can't with estrone and estradiol. Could 5α-reduction of these androgens influence the current findings? It would appear that, with the exception of granulosa-luteal cell studies, no assessment of metabolism of androgens other than 17-ketosteroid oxidoreductase was made. How much does Dr. Barbieri think other metabolism of these androgens influenced his results?

Second, Dr. Barbieri is careful to point out that the maximum velocity of this enzyme was not assessed in any of these experiments. This is too bad because maximum velocity is a constant for any enzyme that allows for comparisons between tissue or cell fragments. If maximum velocity is not measured, then interpretation of data is problematic. Incubation conditions can influence findings leading to potential errors of interpretation. We look forward to Dr. Barbieri's future studies of maximum velocity, which hopefully will confirm the present results.

Last, Dr. Barbieri has estimated 17-ketosteroid oxidoreductase activity indirectly by measuring the ratios of testosterone to androstenedione and non–sex hormone–binding globulin–bound testosterone to androstenedione in the circulation of normal and hyperandrogenized women. This type of comparison would be reasonable if the metabolic clearance rates of androstenedione and testosterone were similar in normal and hyperandrogenized women.[1] For androstenedione this is the case, but for testosterone there is usually a twofold increase of its metabolic clearance rate in hyperandrogenized women as compared with normal women. Dr. Barbieri did not measure the metabolic clearance rate of testosterone or use historic data to control for this potential artifact. This deficit is partially accounted for by measuring non–sex hormone–binding globulin–bound testosterone levels but not completely. Thus the difference in the ratios of circulating testosterone or non–sex hormone–binding globulin–bound testosterone to androstenedione probably underestimates the actual activity of this enzyme to form testosterone in vivo.

Although as yet it is not definitive, this article represents a considerable amount of work. The methods used show an admirable degree of sophistication and the restraint exercised in interpreting the results demonstrate a clear understanding of the limits of this study.

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Dr. Robert B. Jaffe, San Francisco, California. As always, Dr. Barbieri has given an extremely lucid and thoughtful presentation of a large amount of data. I would suggest that to localize the enzyme, when the probes for either of the two forms that he listed on the board become available to him or when the expressed protein from these genes becomes available, he consider using either in situ hybridization with the former or immunocytochemical localization with the latter as a much better way to determine precisely where the enzymes are located.

This is particularly true for his luteinizing granulosa cells because these represent a very diverse group of cells. I suspect it might be better if you were to use intact ovarian tissue with in situ hybridization or immunocytochemical localization as a better way to get at the site of production of these enzymes.

Dr. Edward E. Wallach, Baltimore, Maryland. Since the ovarian stroma merges with the theca cells surrounding the follicle and the theca cells are responsible for production of androstenedione as a precursor or substrate for estradiol specifically in the preovulatory phase of the cycle, I wonder if you would comment on whether the ratio described may be dependent, in either the hyperandrogenic women or the nonhyperandrogenic women, on the phase of the cycle in which the stromal tissue was obtained.

Dr. Georgeanna S. Jones, Norfolk, Virginia. I was particularly interested in the location of the androgenic and the estrogenic enzyme. It seems to me that in the normal situation of these enzymes may allow us to understand how under abnormal conditions we see an apparent incongruous elevation of luteinizing hormone.

Dr. M. Yusoff Dawood, Houston, Texas. Dr. Barbieri, could you tell us how you calculated your non–

sex hormone–binding globulin–binding globulin? What method did you use? Have you had a chance to examine this enzyme in either corpus luteum or ovarian tissues in women treated with gonadotropin-releasing hormone agonists or oral contraceptives?

Dr. James M. Roberts, San Francisco, California. I have two questions. One is related to the affinity of the enzyme. A linear increase in production of hormone going from 200 mol/L to 500 mol/L in the animal would imply the Michaelis-Menten coefficient is probably 10 times higher. Is that a reasonable value for this enzyme?

Second, as an extension of Dr. Jaffe's comment and perhaps a way you can get more information out of the data you've generated, I'm concerned about expressing enzyme activities per milligram protein in cytosol and membrane when you have different size cells, and I wonder, if you looked at total activity per cell, by coming up with some estimate on how many total milligrams of protein are in the membranes from cells and how many total milligrams of protein are in cytosol, whether the differences would still maintain.

Dr. W. Leroy Heinrichs, Stanford, California. It's an important study; this ubiquitous enzyme seems, however, at least in the endometrium to be regulated by progesterone. You've chosen as your two patient groups normally cycling women and hyperandrogenic women, the latter unlikely to be ovulating and therefore with lesser concentrations of progesterone in the circulation.

My questions are, have you evaluated your enzyme in terms of the progesterone environment in the preovulatory-postovulatory portions of the cycles of normal women and what are the progesterone concentrations in the tissue or plasma of women who are hyperandrogenic?

Dr. Donald Pittaway, Winston-Salem, North Carolina. One difficulty I see is when one looks at the ovarian venous data in such women. In general, the testosterone-to-androstenedione ratios are actually decreased in the androgenic women. Certainly there are exceptions to this. The majority of women actually have a reduced ratio in the ovarian veins, indicating a less efficient glandular conversion of androstenedione to testosterone. I wonder if you would comment on this.

Dr. Norman F. Gant, Dallas, Texas. I believe that Roberts asked one of the key questions and that was what was the specific activity per cell? If we're talking about hyperandrogenism, are we talking about more cells, bigger cells or both? How long have these cells been "banged away at" by luteinizing hormone? The key issue is whether the specific activity of this enzyme is increased or whether there are more or larger cells.

Dr. Frederick Naftolin, New Haven, Connecticut. In support of Dr. Barbieri's work, we've used such a probe as Dr. Jaffe suggests. We have an antiserum grown from Lou Engle's oxidoreductase 20 years ago. In fact, what we find is that there is a scattered patchiness in the stroma that registers immunopositive for this enzyme. There are, in fact, two enzymes, and our antiserum only recognizes the one that's in the placenta and not the one, for example, in the uterus.

I would say that it would be very helpful for you to use such an antiserum as a control on the dissections you use to get the stroma, because I believe many of the questions that we're asking relate to the differences within the stroma in the ability of this enzyme to be expressed.

Dr. Barbieri (Closing). I would like to thank Dr. Judd for his thorough review of my manuscript. Because of time constraints, I was unable to mention that the work of Dr. Judd played an important role in the development of my ideas concerning 17-ketosteroid oxidoreductase.

In menopausal women the ovary consists mainly of ovarian stroma. Dr. Judd has demonstrated that the main steroid secreted by the menopausal ovary is testosterone. I believe that in the menopause high luteinizing hormone levels activate stromal 17-ketosteroid oxidoreductase, causing an increased conversion of androstenedione to testosterone. As in the menopausal women, the high luteinizing hormone concentration present in hyperandrogenic women may cause an activation of ovarian stromal 17-ketosteroid oxidoreductase.

I would like to also say that this article in no way represents the final data concerning this subject. I see science as a cumulative process, and my goal with this article was to stimulate other researchers to try to describe in greater detail the microheterogeneity that might exist with regard to ovarian 17-ketosteroid oxidoreductase.

Dr. Judd raises an important point concerning the alternative pathways for metabolism of androstenedione, testosterone, estrone, and estradiol in our laboratory studies. In the granulosa cells we incubated radioactive estradiol and radioactive progesterone with the cells for 24 hours and then evaluated the radioactive metabolites with high-performance liquid chromatography. In the human granulosa-luteal cells, very little of the added estradiol or progesterone was metabolized to other identifiable metabolites. In the ovarian stroma, our analysis of possible routes of metabolism for androstenedione and testosterone was more limited. We did analyze tissue for the presence of androsterone and androstanediol and were unable to detect significant amounts of these metabolites in the medium of incubation of ovarian stroma. Small amounts of dihydrotestosterone, <5 pg/mg wet weight of tissue per 48 hours, did accumulate in the media. So this is an important concern that would need further analysis.

Another question by Dr. Judd concerns the determination of the maximum velocity and Michaelis-Menten coefficient. It's very difficult to obtain enough human tissues for 17-ketosteroid oxidoreductase to perform these studies. In the laboratory I prefer to work with rat tissues rather than human tissues because of tissue availability. Previous work in our laboratory, in conjunction with Dr. Ryan, has demonstrated that there appears to be preferential affinity of the rat gran-

ulosa cell 17-ketosteroid oxidoreductase for estrone and preferential affinity of the rat Leydig cell 17-ketosteroid oxidoreductase for androstenedione; and in those articles we did publish detailed maximum velocity and Michaelis-Menten coefficient values.

The third point by Dr. Judd was that in hyperandrogenic women there is an increased metabolic clearance rate of testosterone, and I agree with him that this would tend to produce an error of underestimation of the ratio of serum testosterone to androstenedione. Our data suggest that the ratio of serum testosterone to androstenedione is increased in our population of hyperandrogenic women compared with controls. Dr. Jaffe mentioned using immunocytochemical techniques to look at the localization of the 17-ketosteroid oxidoreductase enzyme, and I agree that needs to be performed at a future date.

Dr. Wallach questioned the effects of cycle phase on 17-ketosteroid oxidoreductase, and we have no data concerning the effects of progesterone, or cycle phase, on either granulosa cell or stromal 17-ketosteroid oxidoreductase. Of interest, as he mentioned, the theca provides a substantial amount of androstenedione for aromatization by the granulosa cells to estrone. A characteristic feature of follicular fluid is that there is a very high ratio of estradiol to estrone, but the ratio of testosterone to androstenedione is very, very low. So the androstenedione is present in high concentrations, and estradiol is present in high concentrations but not testosterone.

Dr. Jones mentioned the possibility that activation of 17-ketosteroid oxidoreductase may play a role in the control of luteinizing hormone levels in women with hyperandrogenism, and I think this deserves further exploration.

A question was raised concerning our ammonium sulfate precipitation technique for determining non–sex hormone–binding globulin–bound testosterone levels. Sex hormone–binding globulin affinity for testosterone is temperature dependent. The typical ammonium sulfate assays are performed at 4° C, which produces an incorrect assessment of the true non–sex hormone–binding globulin–bound testosterone that actually occurs at 37° C. However, the ammonium sulfate technique is easy to perform and has a low intraassay and interassay coefficient of variation. We calculated the amount of radioactive testosterone bound to the globulin fraction of the ammonium sulfate fractionation and the amount that was in the nonglobulin fraction. These percentages were then multiplied by the total testosterone concentration to arrive at a non–sex hormone–binding globulin–bound testosterone and sex hormone–binding globulin–bound testosterone concentrations.

In the corpus luteum 17-ketosteroid oxidoreductase is probably in the cytosol. I believe Dr. Pittaway has published previous reports on the occurrence of corpus luteum 17-ketosteroid oxidoreductase in the cytosol.

Dr. Roberts raises the question of the Michaelis-Menten coefficient. I believe that the Michaelis-Menten coefficient for the 17-ketosteroid oxidoreductase with estrone used as a substrate and the enzyme derived from the granulosa cell or placenta is in the range of approximately 1 to 4 μmol/L. We chose subtrate concentrations slightly below this because we believe this is the concentration of substrate that exists in the ovary in the in vivo state.

Dr. Roberts and Dr. Gant raised the issue of activity per whole cell. I think in some way the hyperthyroidism situation is analogous to the situation of hyperandrogenism. In hyperthyroidism the thyroid overproduces thyroxine and triiodothyronine, but the degree of overproduction of triiodothyronine is much greater than the degree of overproduction of thyroxine. So the thyroid becomes more efficient in producing the biologically potent thyroid hormone. In a like manner, in the hyperandrogenic ovary there is a dramatic increase in both androstenedione and testosterone production, but, especially in women with ovarian stromal hyperthecosis, the increase in testosterone production is disproportionately greater than the increase in androstenedione production.

Dr. Heinrichs raised the issue of endometrial 17-ketosteroid oxidoreductase being regulated by progesterone. I think this nice example of regulation of an enzyme by progesterone suggests that the upstream region from 17-ketosteroid oxidoreductase should be cloned, and the regulation of this gene should be looked at in greater detail. A progesterone hormone response element is probably present upstream of the 17-ketosteroid oxidoreductase gene.

The serum concentration of testosterone and androstenedione were measured in the follicular phase. The progesterone concentrations in hyperandrogenic and control women were <3 ng/ml.

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DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS,** section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40–49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970s—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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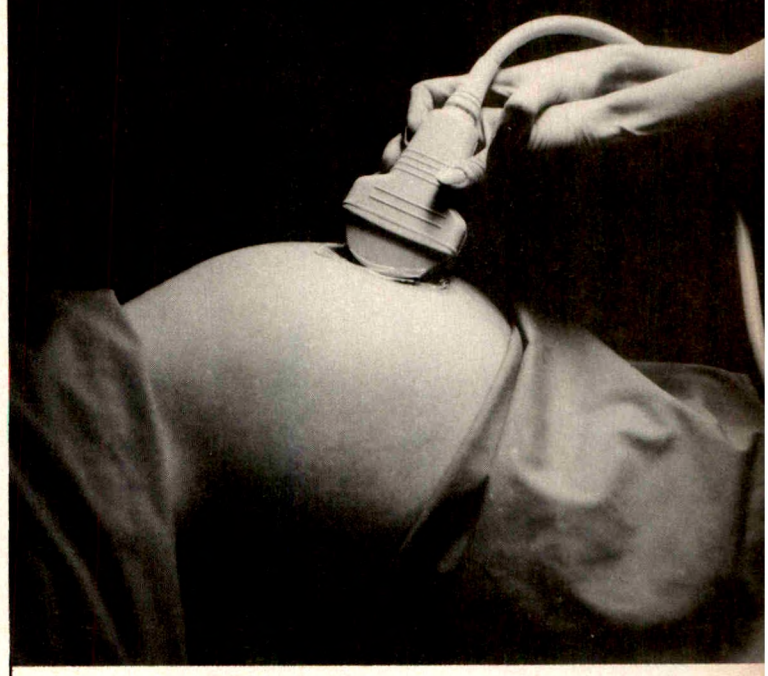
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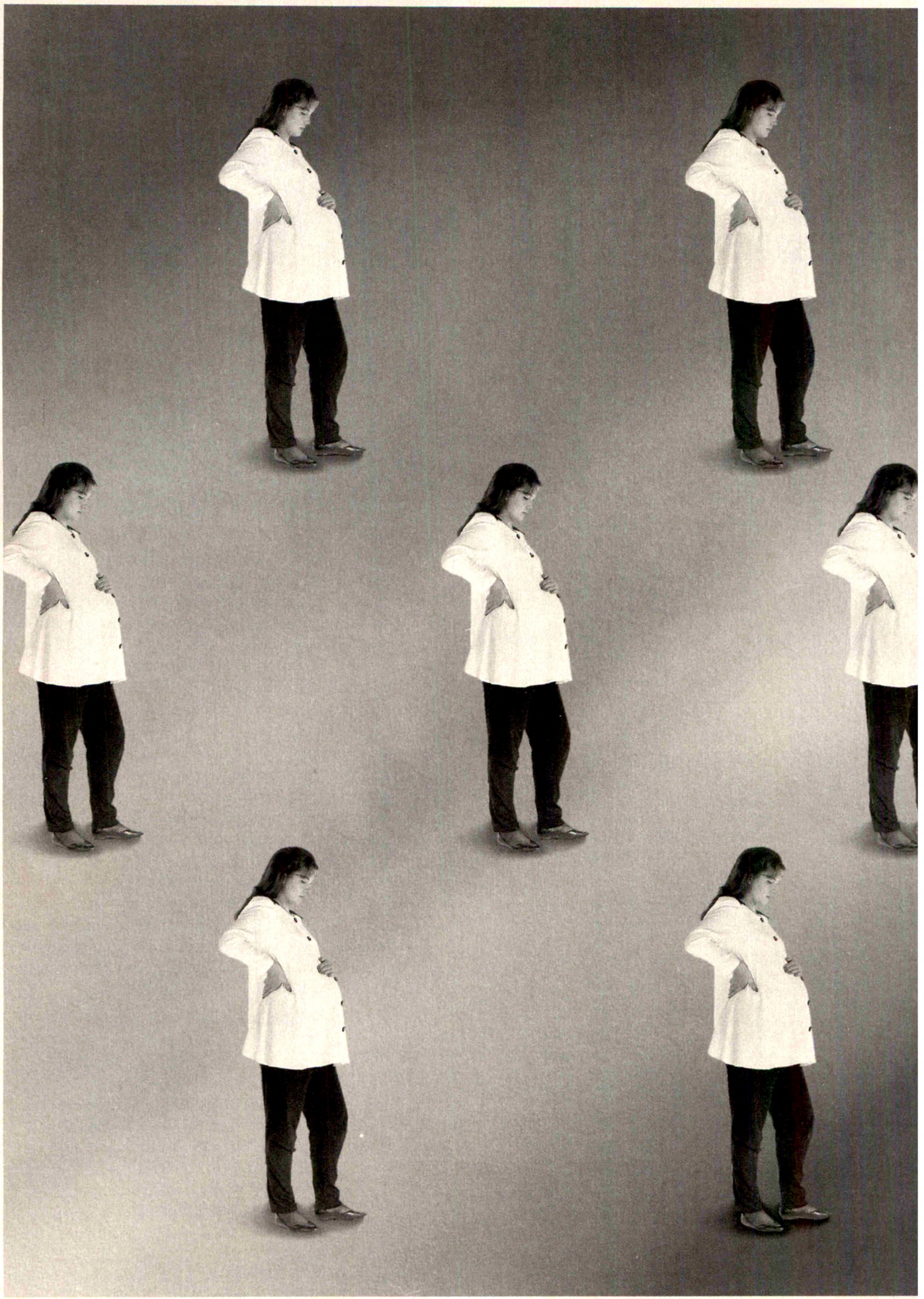
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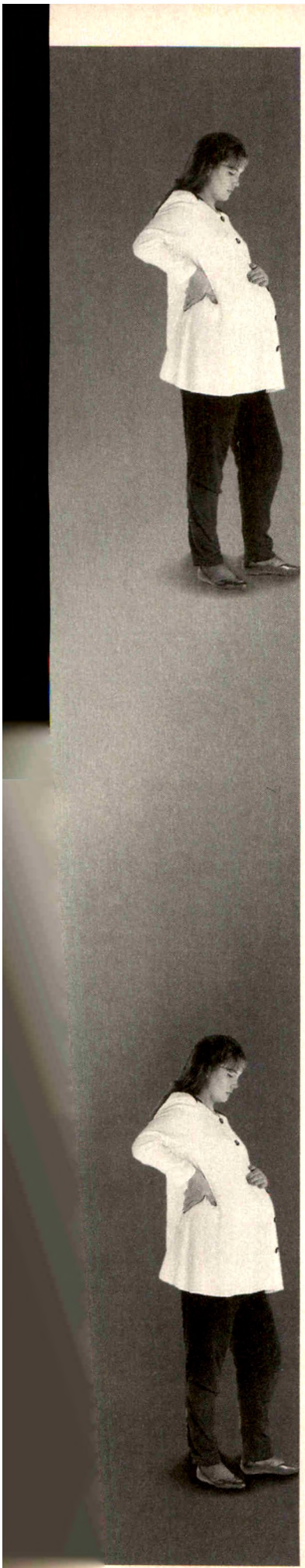
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Anticonvulsant effects of magnesium sulfate on hippocampal seizures: Therapeutic implications in preeclampsia-eclampsia

David B. Cotton, MD, Cynthia A. Janusz, BS, and Robert F. Berman, PhD
Detroit, Michigan

OBJECTIVE: The objective of this study was to determine whether magnesium sulfate has central anticonvulsant effects.
STUDY DESIGN: In three experiments we investigated the anticonvulsant properties of magnesium sulfate on the hippocampus because of its high density of *N*-methyl-D-aspartate receptors and link to clinical epilepsy. Seizure activity was elicited in the hippocampus of rats or in in vitro hippocampal brain slices. The effects of magnesium on seizure activity were determined.
RESULTS: (1) Intraperitoneal magnesium sulfate (270 or 360 mg/kg) failed to block hippocampal seizures but reduced electroencephalographic amplitude and seizure duration. (2) Injection of magnesium sulfate directly into a seizure focus blocked seizures and elevated seizure thresholds. (3) Low magnesium levels produced epileptiform activity in an in vitro hippocampal brain slice preparation; the epileptiform activity was reversed by increasing the magnesium concentration.
CONCLUSIONS: Magnesium sulfate has central anticonvulsant activity on hippocampal seizures, implicating the *N*-methyl-D-aspartate receptor in eclamptic seizures and in the therapeutic efficacy of magnesium sulfate. (AM J OBSTET GYNECOL 1992;166:1127-36.)

Key words: Magnesium sulfate, hippocampus, seizures, eclampsia

Magnesium sulfate has been used for >60 years to prevent and treat eclamptic seizures.[1-3] In spite of magnesium sulfate's empiric success in obstetrics, the literature of other medical disciplines has decried its use on the grounds that it does not have any central inhibitory action and merely masks seizures via peripheral neuromuscular blockade.[4-7] However, on the basis of the well-documented clinical response of eclamptic seizures to magnesium sulfate in the absence of any neuromuscular blockade, Pritchard[8] has proposed that magnesium sulfate does have central effects.

Experimental work in dogs, cats, and subhuman primates also provides support for a central mechanism of action. Borges and Gücer[9] showed that intravenous magnesium sulfate infusion suppressed neuronal burst firing and decreased interictal spike frequency in a penicillin-induced epilepsy model. Suppression occurred at serum magnesium levels well below those required for complete neuromuscular blockade; Borges and Gücer concluded that magnesium sulfate does have a central effect.

In a similar model of penicillin-induced epileptic foci in cats Koontz and Reid[10] were unable to duplicate Borges and Gücer's[9] findings. In Koontz and Reid's model a saline-infused group had the same rapid decrement in seizure activity as the group receiving magnesium sulfate. Koontz and Reid[11] later performed an experiment with the same seizure model to determine the effect of pretreatment with magnesium sulfate; again, they could not document any anticonvulsant effect of magnesium sulfate. They concluded that parenteral magnesium sulfate had no demonstrable anticonvulsant effect in the penicillin-induced seizure focus model in cats.

In another experiment, Hilmy and Somjen[12] demonstrated a fourfold to sevenfold greater deposition of magnesium in peripheral tissues as opposed to brain tissues after intravenous magnesium administration. These investigators concluded that magnesium acts primarily through neuromuscular blockade and that a barrier mechanism protects the central nervous system from high levels of magnesium.

Others have suggested that in eclampsia any apparent central effects of magnesium sulfate must be the result of disruption of the blood-brain barrier and leakage of magnesium into the brain.[4, 10] Donaldson[4] expanded on these observations and concluded that magnesium sulfate does not treat eclamptic convulsions and adds substantial risks to mother and baby.

Because of these disparate conclusions regarding magnesium sulfate's mechanism of action, we examined in rats the effects of magnesium sulfate on seizures initiated in the hippocampus, a brain region with a low seizure threshold and a high density of *N*-methyl-D-

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aspartate receptors. A study of hippocampal seizures should provide a sensitive index of the possible central nervous system effects of magnesium sulfate. The high density of *N*-methyl-D-aspartate receptors is also of interest because these receptors have been linked to various models of epilepsy and are blocked by magnesium.[13]

Material and methods

Surgical preparation. Male Long-Evans rats (Harlan Sprague-Dawley Inc., Indianapolis) were individually housed in stainless steel cages in an environmentally controlled vivarium under 12-hour light-dark cycles. Animals had free access to food throughout the experiments. Twelve animals (275 to 300 gm) were surgically anesthetized (60 mg/kg sodium pentobarbital intraperitoneally) and stereotaxically implanted with chronically indwelling bipolar electrodes (MS-303/2, Plastics One Inc., Roanoke, Va.) aimed for the dorsal hippocampus. The stereotaxic coordinates were 4.0 mm posterior bregma, 2.0 mm lateral from midline, and 4.0 mm ventral to the superior surface of the level skull. The electrode assembly was chronically attached to the skull with three stainless steel jeweler's screws and acrylic cement. Animals were allowed 1 week of postsurgical recovery before further treatments were begun. An additional group of 5 male Long-Evans rats (275 to 300 gm) were surgically implanted with chemitrodes into the dorsal hippocampus as described above. The chemitrode, which permitted electric stimulation and intracranial drug injection at the same brain site, consisted of a bipolar electrode (MS303/3, Plastics One Inc.) cemented to a 23-gauge stainless steel cannula. A 30-gauge obturator plugged the cannula until the time of injection. The ends of the electrode extended 0.5 mm past the end of the cannula. The coordinates for implantation were the same as those described above. A 1-week postsurgical recovery period was again used. A final group of 10 male Long-Evans rats (200 to 260 gm) were used for the in vitro hippocampal brain slice experiments as described below.

Experimental design and protocol

Experiment 1. In this experiment the effects of intraperitoneal injections of magnesium sulfate at 270 and 360 mg/kg on hippocampal epileptiform afterdischarges were determined. Rats implanted with bipolar electrodes in the dorsal hippocampus were placed into a Plexiglas acrylic plastic recording chamber and leads from a polygraph (model 7, Grass Instruments Co., Quincy, Mass.) were attached to the implanted electrodes. Baseline electroencephalographic activity was recorded from the hippocampus via the implanted electrodes. One-second trains (100 Hz, 1-millisecond pulse duration, biphasic, square waves) of low-intensity electric stimulation were delivered through the implanted electrodes beginning at a current intensity of 10 μA. Stimulation intensity was increased in steps of 10 μA until a brief, 5- 10-second epileptiform afterdischarge was recorded from the hippocampus immediately after stimulation. The level of stimulation that evoked an afterdischarge was defined as the afterdischarge threshold; this threshold was used for all drug studies. Electric stimulation was delivered by a stimulator (model S88, Grass Instruments) equipped with two constant-current units (Photoelectric Stimulus Isolation Unit, constant current output, Grass Instruments). An oscilloscope (502A, Tektronix, Inc., Portland, Ore.) was used to monitor the amplitude of the electrical stimulation. Twenty-four hours after afterdischarge threshold determination, the animals were returned to the recording chamber. Three to 5 minutes of baseline electroencephalogram was first recorded, with recording continuing throughout the session. Animals were then injected intraperitoneally with either 270 or 360 mg/kg magnesium sulfate or a volume of saline solution equal to that used for the 360 mg/kg dose on one of three test days. The magnesium sulfate doses were chosen on the basis of an earlier study that reported the anticonvulsant effects of magnesium sulfate in rats.[14] The order of drug administration was randomized across days. Ten minutes after injection animals were stimulated through the implanted electrodes at the afterdischarge threshold current. Epileptiform afterdischarge duration, frequency and electroencephalographic amplitude before and after the seizure were recorded. Finally the duration of postictal electroencephalographic depression was determined. All drug injections were separated by a minimum of 48 hours. Magnesium sulfate was made up in sterile saline solution at a concentration of 90 mg/ml.

Serum magnesium levels were measured immediately before and at timed intervals after intraperitoneal injection of magnesium sulfate in 10 animals. Each rat was surgically anesthetized and implanted with a chronic, indwelling jugular catheter. The catheters were constructed of polyethylene tubing, filled with heparinized saline solution to reduce obstruction of the cannula, and sealed with a metal pin. Catheters were flushed twice daily with heparinized saline solution, and blood samples were taken 3 days after surgery. In brief, rats were injected intraperitoneally with either 180, 270, or 360 mg/kg of magnesium sulfate. At 0, 5, 15, 30, or 60 minutes after injection the pin was removed from the catheter and an 18-gauge needle and syringe inserted into the end of the catheter. Then 0.5 ml samples of blood were collected in 1 ml Microtainer vials (Becton-Dickinson, Rutherford, N.J.). Magnesium levels in 0.7 μl serum samples were analyzed colorimetrically (Eastman Kodak Co., Rochester, N.Y.).

Experiment 2. Direct intracranial injections of magnesium sulfate were made into the dorsal hippocampus through the chronically implanted chemitrodes to determine the effects of magnesium sulfate on seizure activity directly at the seizure focus. In brief, the afterdischarge threshold was determined for these animals as described for experiment 1. Again, an electroencephalographic recording was taken continuously throughout this experiment. Animals were then injected with 1 μl of saline solution or 1 μl of saline solution containing an empirically chosen dose of 50 μg of magnesium sulfate.[15] Five minutes after chemitrode injection animals were stimulated at the afterdischarge threshold, and the afterdischarge duration and spike frequency were determined. Chemitrode injections were made with a 10 μl microsyringe (Hamilton Co., Reno, Nev.) attached to a 30-gauge injection needle cut to fit the implanted chemitrode cannula.

Injections were made over a 60-second period; the injection needle was left in place an additional 60 seconds to ensure drug delivery to the hippocampus as previously described.[15] If drug injection blocked the afterdischarge, current levels were raised in steps of 10 μA until an afterdischarge was elicited.

Experiment 3. Ten rats (200 to 260 gm) were decapitated and their brains quickly removed. The brain was placed on its dorsal surface; the hippocampus was rapidly dissected on ice at 4° C as previously described.[16] The hippocampus was placed onto a tissue slicer (Stoelting Co., Wood Dale, Ill.), and 400 μm slices were cut at a 70 degree angle perpendicular to its septotemporal axis. Slices were immediately placed into cold, oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid consisting of 2.4 mmol/L calcium chloride, 1.3 mmol/L magnesium sulfate, 5 mmol/L potassium chloride, 120 mmol/L sodium chloride, 1.24 mmol/L sodium biphosphate, 26 mmol/L sodium bicarbonate, and 10 mmol/L dextrose. The magnesium-free medium was prepared by omitting magnesium sulfate. All slices were transferred to artificial cerebrospinal fluid within 7 minutes from the time of death to ensure viability of the tissue. Slices were gently transferred to the recording chamber. The recording chamber was constructed of transparent Plexiglas acrylic plastic. The atmosphere within the chamber was humidified, oxygenated, and heated to 34° C. A brain slice well on which nylon netting was stretched was placed into the chamber and contained 2 ml of artificial cerebrospinal fluid. Hippocampal slices were placed on the netting. Under these condition stable electrophysiologic activity can be maintained in brain slices for 8 to 12 hours. Slices were allowed to equilibrate to the chamber for 1 hour before electrophysiologic recordings were begun. Extracellular recordings were made with glass micropipettes filled with 2 mol/L sodium chloride and with impedances between 1 to 4 megohms. The tip of the recording electrode was placed into the pyramidal cell layer of the CA3 subregion of the hippocampus at a depth of approximately 70 to 100 μm with a hydraulic micropositioner (model 650, David Kopf Instruments, Wood Dale, Ill.). A concentric bipolar, tungsten microstimulating electrode was used to antidromically stimulate the Schaffer collateral axons in the stratum radiatum of CA3. Extracellular field potentials recorded from pyramidal cells in CA3 were amplified 1000-fold (model DAM-50, World Precision Instruments, New Haven, Conn.), visualized on a digital storage oscilloscope (model 4094C, Nicolet, Madison, Wisc.) and stored on disk for later analysis. Electric stimulation (single 0.1-millisecond monophasic square-wave pulses, 5 to 30 V) was delivered by a stimulator (model S-48, Grass Intruments) and PSIU-6 stimulus isolation unit. Stimulation of the Schaffer collateral fibers was delivered every 60 seconds, and the intensity was adjusted until a stable extracellular field potential was obtained.

After 15 to 20 minutes of superfusion with normal artificial cerebrospinal fluid (i.e., magnesium-containing), magnesium-free artificial cerebrospinal fluid was introduced. Evoked field potentials continued to be recorded every 60 seconds. After 20 to 25 minutes in a magnesium-free artificial cerebrospinal fluid magnesium was added back to the superfusion solution at a concentration of 90 and then 180 μmol/L. The occurrence of epileptiform bursting under magnesium-free conditions was recorded, as were the effects of the readdition of magnesium sulfate.

Histology. At the end of experiments 1 and 2 animals were injected with an overdose of sodium pentobarbital (120 mg/kg intraperitoneally) and perfused transcardially with 0.9% saline solution followed by 10% formalin. The brains were then frozen-sectioned at 20 μm, mounted on microscope slides, and stained with cresyl violet. The precise locations of the implanted electrodes and chemitrodes were then verified microscopically under 40× magnification.

Data analysis. Data were analyzed by a one-way analysis of variance. Post hoc testing was performed when appropriate with the Newman-Keuls test. Values are expressed as mean ± SEM. The significance level was set at $p < 0.05$.

Results

Experiment 1. Intraperitoneal injections of magnesium sulfate produced moderate levels of ataxia, muscular weakness, and lethargy at 270 mg/kg, with severe effects seen at the 360 mg/kg dose. The onset of muscular weakness was within 2 minutes after injection, and animals typically showed complete recovery within 40 to 60 minutes.

The effects of intraperitoneal injections of magne-

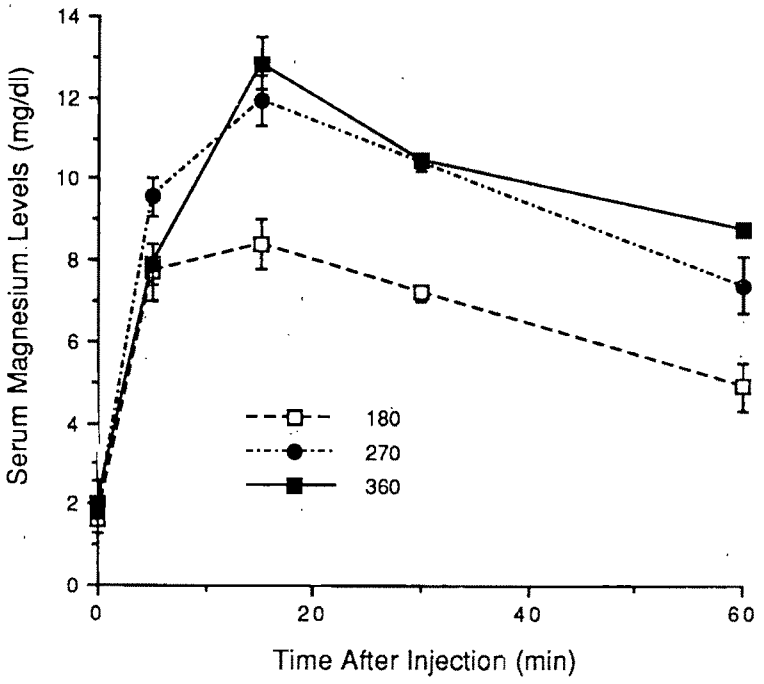


Fig. 1. Serum magnesium levels over a period of 1 hour after intraperitoneal injection of 180, 270, or 360 mg/kg of magnesium sulfate.

Table I. Summary of effects of intraperitoneal injections of magnesium sulfate on hippocampal electroencephalographic seizure threshold, seizure duration, and hippocampal electroencephalographic amplitude

Group	*After discharge threshold (μA)*	*After discharge duration (sec)*	*Electroencephalographic amplitude (% baseline)*
Saline solution (n = 11; mean ± SEM)	72 ± 27	34.6 ± 5.6	100
Magnesium sulfate 270 mg/kg (n = 11; mean ± SEM)	109 ± 40	24.7 ± 5.6	84.6 ± 3.6*
Magnesium sulfate 360 mg/kg (n = 11; mean ± SEM)	90 ± 27	19.0 ± 2.9*	69.1 ± 3.6*

*$p < 0.05$ versus saline-injected control.

sium sulfate on hippocampal afterdischarges are shown in Table I. As shown in the table, magnesium sulfate injections did not affect the afterdischarge threshold for producing hippocampal afterdischarges. All animals showed afterdischarges at or near their individual afterdischarge thresholds regardless of the presence of magnesium sulfate. However, the duration of the afterdischarge was significantly reduced after 360 mg/kg of magnesium sulfate compared with saline-injected controls. In addition, electroencephalographic amplitude was significantly reduced when measured 10 minutes after magnesium sulfate injection but before electric stimulation was administered.

The serum magnesium levels after intraperitoneal injection of either 180, 270, or 360 mg/kg of magnesium sulfate in 10 rats are illustrated in Fig. 1. Values are expressed as mean ± SEM. As shown, mean baseline serum magnesium levels were 1.76 mg/dl (0.72 mmol/L). After magnesium sulfate injection there was a rapid rise in serum magnesium levels, which peaked at approximately 15 minutes, followed by a gradual decrease thereafter. The peak elevation in serum magnesium was 12.8 mg/dl (5.28 mmol/L) for the 360 mg/kg dose measured 15 minutes after injection. These serum magnesium levels are similar to those previously reported in rats receiving the same dose of magnesium sulfate.[14]

Experiment 2. The effects of direct chemitrode injections of magnesium sufate into the hippocampus on hippocampal electrographic activity are demonstrated in Fig. 2, which shows a dramatic reduction in the electroencephalographic amplitude 5 minutes after injection of 50 μg of magnesium sulfate (Fig. 2, *B*) compared with a comparable saline injection (Fig. 2, *A*). These



Fig. 2. Representative electroencephalographic tracings recorded from hippocampus 5 minutes after 1 μl saline injection (**A**) or 50 μg injection of magnesium sulfate (**B**) into a hippocampal seizure focus, demonstrating the marked reduction in amplitude produced by magnesium sulfate.

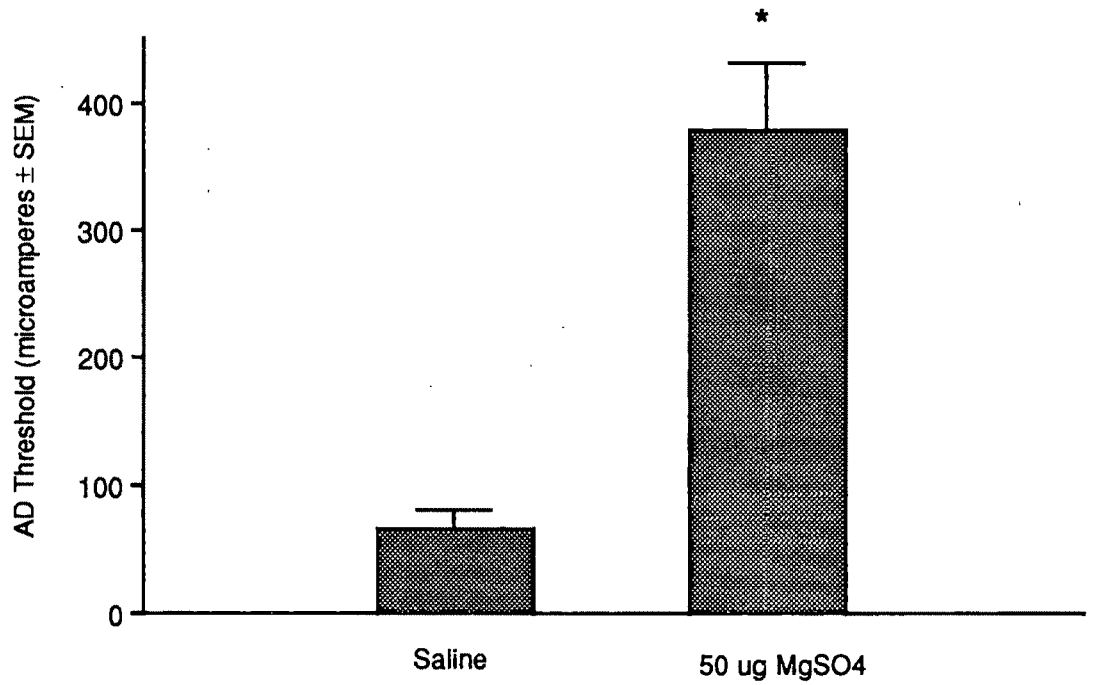


Fig. 3. Increased hippocampal afterdischarge *(AD)* threshold after magnesium sulfate *($MgSO_4$)* injection into a hippocampal seizure focus. *Asterisk*, $p < 0.05$ versus saline control injections.

effects were evident within 90 seconds of injection. Animals were still during most of the period of electroencephalographic quiescence but did not exhibit muscular weakness, ataxia, or any obvious signs of hypermagnesemia. The effects of direct injections of magnesium sulfate into a hippocampal seizure focus on the afterdischarge threshold are shown in Fig. 3. Injections of 50 μg significantly ($p < 0.05$) elevated the threshold for producing a hippocampal afterdischarge. Representative electroencephalography records from one set of injections are shown in Fig. 4. The afterdischarge threshold after a 1 μl saline injection was 26 μA, as shown in Fig. 4, *A*. No afterdischarge was elicited 5 minutes after a 50 μg injection of magnesium sulfate (Fig. 4, *B*) when stimulation was delivered at the 26 μA afterdischarge threshold. A stimulation current of 287 μA was required to produce an afterdischarge (Fig. 4, *C*).

Experiment 3. Epileptiform bursting of region CA3 pyramidal cells after Schaffer collateral stimulation was observed in all hippocampal brain slices beginning 10 to 15 minutes after the beginning of magnesium-free superfusion. A representative epileptiform burst is demonstrated in Fig. 5, *A*. The average burst duration was 64.5 ± 2.8 milliseconds. Suppression of bursting after the addition of magnesium sulfate to a level of

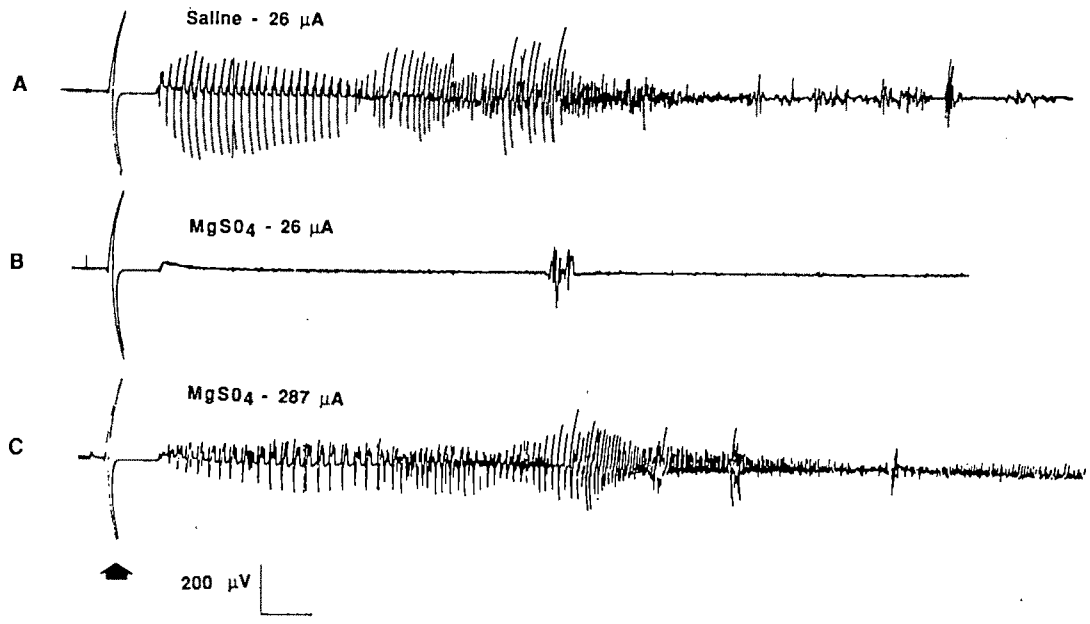


Fig. 4. Representative electroencephalographic records taken immediately after electric stimulation at 26 μA afterdischarge threshold following saline (**A**) or 50 μg magnesium sulfate ($MgSO_4$, **B**) chemitrode injections directly into a hippocampal seizure focus. Note in **B** the absence of afterdischarge at afterdischarge threshold after magnesium sulfate injection. It was necessary to increase stimulation intensity to 287 μA to evoke afterdischarge in this animal as shown in **C**. *Arrow*, Offset of 1-second pulse of electric stimulation.

1.3 mmol/L in the superfusion media is demonstrated in Fig. 5, *B*. In some instances bursting (Fig. 5, *C*) was not totally suppressed by magnesium sulfate (Fig. 5, *D*). Bursting could typically be reinstated by again perfusing the slice in magnesium-free artificial cerebrospinal fluid as shown in Fig. 5, *E*. In all cases the suppression of bursting was relatively rapid, occurring within 10 minutes of addition of magnesium sulfate to the superfusion.

Comment

Our results lead us to conclude that magnesium sulfate does have central inhibitory effects. This conclusion was supported by our experiment in which parenteral injections of magnesium sulfate reduced electroencephalographic amplitude and seizure duration after electric stimulation. Additionally, that a direct intracranial injection of magnesium sulfate could completely block activity of a hippocampal seizure focus also supports this conclusion.

Electroencephalographic studies in preeclamptic-eclamptic patients undergoing magnesium sulfate therapy have not identified any significant electrocortical changes attributable to magnesium sulfate.[17] A possible explanation for our differing results is that hippocampal activity may not be reflected in routine electroencephalographic recordings. Although the hippocampus has a low seizure threshold, changes in electrophysiologic activity produced by magnesium sulfate would be difficult to document without direct subcortical recordings from this structure.

Although we have demonstrated a central anticonvulsant effect of magnesium sulfate in this model, we have not addressed the question of whether magnesium sulfate crosses the blood-brain barrier. In our preparation the placement of electroencephalographic electrodes directly into the hippocampus could cause sufficient disruption of the vasculature to allow magnesium access to the brain interstitium. However, other investigators have carefully evaluated the relationship of serum to cerebrospinal fluid magnesium levels in both human beings and animal models. Thurnau et al.[18] demonstrated that patients with preeclampsia had approximately an 18% increase in cerebrospinal fluid levels after magnesium sulfate therapy. Thurnau et al. concluded that magnesium sulfate does cross the blood-brain barrier in significant amounts during magnesium sulfate therapy.

Oppelt et al.[19] evaluated magnesium levels in the plasma and cerebrospinal fluid of dogs treated with intravenous magnesium sulfate; they concluded that magnesium did cross the blood-brain barrier in small but significant amounts and suggested that an active transport mechanism might be involved. Additionally, Kato and Somjen[20] demonstrated that magnesium significantly depressed neuronal activity in both cortical and spinal cord postsynaptic membranes in cats. These investigators concluded that a rise in magnesium ion

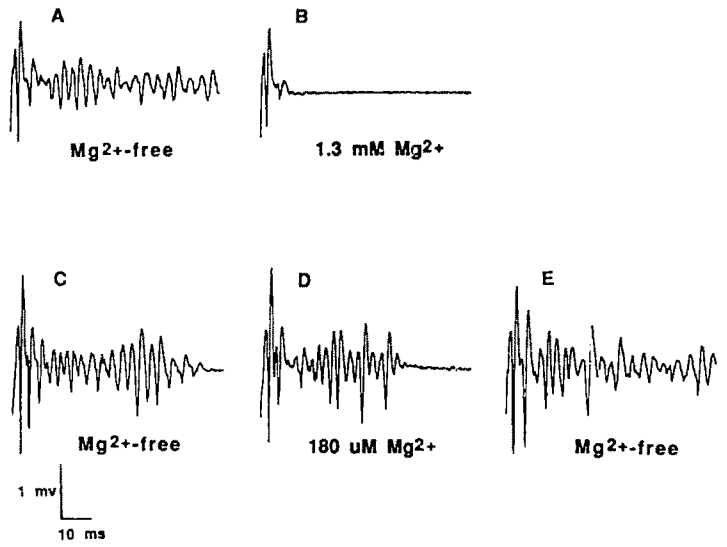


Fig. 5. Representative electrophysiologic field potentials recorded from hippocampal subfield CA3 under magnesium *(Mg)*-free conditions **(A, C)**. Addition of magnesium sulfate completely blocked **(B)** or suppressed **(D)** epileptiform spiking. Spiking could be reinstated by returning slice to magnesium-free conditions **(E)**. Tracings are from two representative hippocampal slices.

concentration in either cerebrospinal fluid or brain by a fraction of the normal levels was sufficient to suppress the activity of central neurons.

In 1974 Chutkow[21] performed a very sophisticated and extensive study of the relationship between cerebrospinal fluid magnesium and the distribution of magnesium in selected regions of the brain during the restoration of magnesium deficits. That study demonstrated that magnesium in cerebrospinal fluid is in equilibrium with an interstitial-cellular pool and suggested that the blood-brain barrier contains an active transport system for magnesium that exhibits regional variation within the brain. Of additional importance is that, although brain magnesium levels did not rise as rapidly as cerebrospinal fluid levels after magnesium replacement, the brain cellular deficits in all areas returned to the normal range within 6 hours of a magnesium injection. In view of those data it appears that even a small increase in the amount of magnesium available to the brain should exert a significant effect.

In spite of our results in this hippocampal model of seizure activity, several other models have failed to demonstrate a central effect of magnesium sulfate. Most of these models, however, have used methods that initiate massive insults to the central nervous system to obtain seizure activity. Choi et al.[22] evaluated the effects of acute changes in plasma magnesium concentration on the threshold for lidocaine-induced seizures in mechanically ventilated rats. Choi et al. hypothesized that magnesium sulfate might be associated with an increased tolerance of the central nervous system to lidocaine toxicity. In that study the lidocaine concentration associated with the onset of electroencephalographic seizures was unaffected by acute hypermagnesemia. This fact led Choi et al. to conclude that the magnesium ion had no anticonvulsant effect. In addition, brain parenchymal magnesium concentrations in that study were similar in the control group receiving saline solution when compared with the magnesium infusion group in spite of a fivefold increase in plasma magnesium levels.

Krauss et al.[23] examined the effects of magnesium sulfate on seizures produced in mice by either maximal electroshock or pentylenetetrazol injection. The electroshock threshold was unaltered by magnesium treatment. Although the magnesium-treated animals had less clonic activity than did the control animals after pentylenetetrazol, the electroencephalographic interictal and ictal seizure discharges were unaffected. The authors attributed the reduction in clonic activity to magnesium blocking the visible manifestations of seizure activity without influencing the neocortical electroencephalographic seizure activity.

In contradistinction to the previously noted studies, Katz et al.[14] recently reported that magnesium sulfate suppressed electroencephalographic seizures induced by hyperbaric oxygen in awake rats. Katz et al. concluded that magnesium sulfate did have a definitive protective action as judged by delaying the electroencephalographic endpoint and that this occurred with an intact blood-brain barrier. Katz et al. suggested that magnesium sulfate might work through calcium-regulated intraneuronal enzymatic processes that alter neuronal excitability and produce seizures. Calcium entry into neurons is regulated by, among other things, specific excitatory amino acid receptor-linked chan-

nels.[24, 25] One type of receptor-channel complex characterized by binding of the excitatory amino acid analog *N*-methyl-D-aspartate has its channel blocked by magnesium ion.[24, 25] Because other blockers of the *N*-methyl-D-aspartate receptor-channel have been shown to be very potent anticonvulsants in seizures induced by maximum electroshock and hyperbaric oxygen, it has been suggested that magnesium sulfate might also act through this mechanism.[13, 14, 26]

We and others have established that low concentrations of magnesium ion can result in epileptiform afterdischarges in hippocampal brain-slice preparations.[27, 28] We chose the hippocampal model of epilepsy because the hippocampus contains one of the highest concentrations of *N*-methyl-D-aspartate receptors of any region in the brain.[29, 30] One of the most likely mechanisms for this seizure activity is activation of the *N*-methyl-D-aspartate receptor. It is possible that in this hippocampal model magnesium sulfate exerted its seizure activity suppression via the *N*-methyl-D-aspartate receptor. On the basis of these data, we speculate that excitatory amino acid receptors such as the *N*-methyl-D-aspartate receptor may play a role in the genesis of eclamptic seizures and may explain, in part, the therapeutic efficacy of magnesium sulfate in this disorder.

We conclude that magnesium sulfate does exert central anticonvulsant effects on hippocampal seizure activity based on data derived from both in vivo and in vitro seizure models.

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Editor's note: This manuscript was revised after these discussions were printed.

Discussion

DR. ALLAN B. WEINGOLD, Washington D.C. (Fellow). Although magnesium sulfate remains the drug of choice in this country for the treatment of preeclampsia, its mechanism of action remains unknown. As a result of long but uncontrolled experience, obstetricians are confident of its efficacy and safety. Alternatives to magnesium sulfate are thought to present added risks to mother, fetus, or both.

Yet the treatment of seizures by neurologists, neurosurgeons, and internists does not include the use of magnesium sulfate. The inability to establish a pharmacologic mechanism plays a major role in this dichotomy.

One anticonvulsant action is thought to be a result of peripheral paralysis with blockade of myoneural transmission. Yet the recurrence of seizures during magnesium therapy is rare even when plasma levels are well below those required for neural blockade.

Is there a central action? Several years ago Sibai demonstrated that patients with eclampsia had abnormal electroencephalograms: both paroxysmal spike activity and generalized diffuse slowing. These findings were frequent in spite of serum magnesium levels considered therapeutic (5 to 8 mg/dl).

Drs. Cotton and Berman examined in rats the effects of magnesium sulfate on seizures initiated in the hippocampus, a brain region with a low seizure threshold and a high density of *N*-methyl-D-aspartate receptors. The high density of *N*-methyl-D-aspartate receptors is of interest because these receptors have been linked to various models of epilepsy and are blocked by magnesium.

Stereotaxically implanted bipolar electrodes were placed in the dorsal hippocampus in 24 rats. In 12 animals the effect of low-intensity electric stimulation on the electroencephalogram was studied after intraperitoneal placement of magnesium sulfate 270 or 360 mg/kg—a dose that translates to 19 to 25 gm of magnesium sulfate in the 70 kg pregnant female. Severe effects of ataxia, muscular weakness, and lethargy were observed at the 360 mg/kg dose. In spite of this the injections did not raise the threshold for producing hippocampal discharges, although electroencephalographic amplitude was significantly reduced.

In the second experiment, with the same electrodes in place, direct injections of magnesium sulfate into the dorsal hippocampus were studied. There was a significant reduction in the electroencephalographic amplitude and the animals exhibited no peripheral signs of hypermagnesemia.

In 10 additional animals hippocampal slices were monitored by electrophysiologic recordings as magnesium sulfate was added to the superfusion solution. Suppression of bursting occurred with the addition of magnesium sulfate.

Recent evidence supports the concept that cerebral vasospasm is involved in the pathogenesis of eclampsia. Magnesium has been shown to enhance production of prostacyclin by vascular endothelium and to modify vasoactive substances in serum samples of patients with eclampsia by opposing calcium-dependent smooth-muscle constriction. Magnesium prevents cell death in cultured hippocampal neurons after exposure to ischemia, further suggesting a central mechanism.

Granting the importance of Dr. Cotton's data and these additional observations as to pharmacologic action, we must still address the fact that many centers in the United Kingdom, Scandinavia, New Zealand, and Australia treat preeclampsia-eclampsia with benzodiazepine and antihypertensives and have obtained maternal and perinatal mortality rates that are quite competitive with the gold standard set by Pritchard et al. in Dallas.

Questions for Dr. Cotton:

1. My colleagues in neurology tell me that the hippocampus is involved in focal epilepsy and is usually related to some direct trauma, either metabolic or vascular, to the area. As demonstrated by Sibai and others, seizure activity in eclampsia is generally associated with diffuse abnormalities in the electroencephalogram. Is the hippocampal site an appropriate model for demonstrating the effect of magnesium in the prevention of eclamptic seizures?

2. How can one translate the observation that there was inadequate seizure suppression in the rats given intraperitoneal magnesium sulfate at levels producing systemic toxicity to the use of this drug in the pregnant patient in safe, non-central nervous system–suppressing doses?

3. Dr. Cotton has established credentials as an intensivist in our specialty. Does he believe that the continued use of magnesium sulfate in the patient with eclampsia is justifiable in view of the controversy over its pharmacologic activity and the availability of alternative agents of proven efficacy and apparent equal safety?

I thank Dr. Cotton for submitting his article early and commend him for his study.

Dr. Norman F. Gant, Dallas, Texas. Dr. Cotton, I want to tell you how much I appreciated your presentation, and I will personally provide the sword for Dr. Donaldson to fall on.

With respect to Dr. Weingold's comment about comparable results for the treatment of eclampsia in the United Kingdom, in fact, that is not true. I served 3 years ago, as did Fred Zuspan and Leon Chesley, on a work-study section for the Royal College of Obstetricians and Gynecologists; in fact, maternal mortality, a point most of us can use for a definite end point, has not decreased in 25 years in the United Kingdom with respect to treatment for eclampsia.

Dr. Frederick Naftolin, New Haven, Connecticut. I have the same concerns about the model because in human beings epilepsy is apparently the result of a long-term insult that is usually vascular. Most people believe that it starts sometime at birth and results in abnormalities of somatostatin. I think one possibility in interpreting your results is that the magnesium sulfate may have an effect that goes through somatostatin, an excitatory amino acid that apparently is the cause of or is related to the loss of the cells that we see in patients with hippocampal epilepsy. Have you looked to see whether magnesium sulfate changes somatostatin production or secretion by neurons in the hippocampus?

Dr. Robert Goodlin, Placerville, California. This is an interesting study, Dr. Cotton, but I do not understand why magnesium sulfate works only with eclampsia and not other types of seizures.

Dr. Michael Ross, Los Angeles, California. Very nice work, Dr. Cotton. How repeatable is the threshold

determination in an individual animal? Was it tested several times?

Second, how comparable would the tissue levels of magnesium achieved by the direct intracerebral injection be to a systemic infusion of magnesium?

Finally, have you tested any other anticonvulsants that act by means other than *N*-methyl-D-aspartate receptors to see whether your speculation holds up?

Dr. Robert K. Creasy, Houston, Texas. Just one small methodologic question: Did you confirm at autopsy the placement of your stereotaxically placed needles?

Dr. Cotton (Closing): I thank Dr. Weingold for his thoughtful discussion. The answer to the first question regarding whether the hippocampus is an appropriate model for the study of magnesium sulfate's effect on eclamptic convulsions is, I believe, yes. The model we are studying is also used to initiate epilepsy and full tonic-clonic convulsions. The process by which epilepsy is established is called "kindling," in which the progressive development of seizures occurs with repeated, initially subconvulsive, electric stimulation of discrete areas of the brain. Key brain areas used for kindling include the amygdala, hippocampus, and extrapyramidal structures such as the substantia nigra or caudate nucleus. In the first phase of this study we were only trying to establish whether magnesium sulfate had a central effect. In future studies we plan to look specifically at magnesium sulfate's efficacy in preventing tonic-clonic convulsions. We also believe this model may prove to be useful in studying pregnancy's effect on seizure threshold and could be used to compare the relative efficacy of various anticonvulsants.

This study does not provide a clear answer to the question regarding the observation that intraperitoneal magnesium sulfate did not adequately suppress seizure activity and whether this could be translated to use in human beings. Further studies in our laboratory that address different routes of magnesium sulfate administration and whether longer duration of therapy results in enhanced magnesium transfer to the brain and more efficacious seizure suppression may clarify this question. This initial study only addressed whether magnesium sulfate had a central effect.

Finally, Dr. Weingold, I believe the use of magnesium sulfate is not only justified in the treatment of eclampsia, it is the gold standard against which other anticonvulsants must be measured. I believe this study provides additional information to support its use.

The answer to your question, Dr. Naftolin, is that we have not looked at somatostatin production or secretion in the hippocampus. However, your observation is fascinating, and we will certainly consider pursuing that question.

Dr. Goodlin, legend has it that when obstetricians consult in neurointensive care units, magnesium sulfate has been used with limited success. Perhaps the reason the magnesium sulfate has a less-than-stellar reputation outside of obstetrics is not from lack of efficacy but from lack of use.

Dr. Ross, the threshold determinations were repeated on several occasions; in general, we saw very little variation in the amount of electric stimulation required to establish seizure activity in a given animal. I cannot answer your question regarding the levels of magnesium achieved by intracerebral injection as opposed to systemic administration because we did not measure brain tissue magnesium levels, but I suspect that the levels would be much higher with direct injection. Finally, we have not tested any other anticonvulsants, but we certainly will.

Dr. Creasy, at the end of experiments 1 and 2 the animals were killed and perfused transcardially with 0.9% saline solution followed by 10% formalin. The brains were then frozen-sectioned at 20 μm, mounted on microscopic slides, and stained with cresyl violet. The precise locations of the implanted electrodes were then verified microscopically under 40× magnification.

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Comparison of the peritoneal cells elicited by oxidized regenerated cellulose (Interceed) and expanded polytetrafluoroethylene (Gore-Tex Surgical Membrane) in a murine model

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OBJECTIVE: The peritoneal fluid cell responses to the available barriers used to prevent postoperative adhesions, oxidized regenerated cellulose (Interceed) and expanded polytetrafluoroethylene (Gore-Tex Surgical Membrane), were compared.
STUDY DESIGN: Oxidized regenerated cellulose and expanded polytetrafluoroethylene were inserted into the peritoneal cavity of mice and the peritoneal fluid cell number, differential cell count, morphologic type, adherence to plastic, and phorbol-12,13-myristate acetate–stimulated hydrogen peroxide production evaluated. Peritoneal fluid cell attachment to oxidized regenerated cellulose and expanded polytetrafluoroethylene was evaluated by electron microscopy.
RESULTS: Oxidized regenerated cellulose promptly elicited increased numbers of large, morphologically activated peritoneal fluid macrophages that adhered more rapidly and produced more hydrogen peroxide than controls. Expanded polytetrafluoroethylene gradually increased the number of normal-appearing peritoneal fluid macrophages with increased hydrogen peroxide production but normal adherence. Macrophages rapidly attached to both materials in vivo, with oxidized regenerated cellulose being rapidly degraded.
CONCLUSION: Oxidized regenerated cellulose, to a greater extent than expanded polytetrafluoroethylene, elicits an acute peritoneal fluid inflammatory exudate in the mouse, which may contribute to the oxidized regenerated cellulose–induced peritoneal injury and de novo adhesions observed in this model. (Am J Obstet Gynecol 1992;166:1137-49.)

Key words: Oxidized regenerated cellulose, expanded polytetrafluoroethylene

The formation of postoperative adhesions, which occur in 50% to 95% of women undergoing gynecologic surgery, continues to plague the reproductive surgeon.[1] Two mechanical barriers have recently been introduced to interpose between surgically traumatized peritoneal surfaces lying in natural apposition; these barriers allow restitution of the mesothelial layer without the formation of adhesions.[2-9] In previous studies evaluating the impact of the materials used in these barriers, it was noted that oxidized regenerated cellulose (Interceed, Johnson & Johnson Medical, Inc., New Brunswick, N.J.) caused a significant injury to the peritoneum, resulting in the formation of de novo adhesions in a murine paradigm. By contrast, expanded polytetrafluoroethylene (Gore-Tex Surgical Membrane, W.L. Gore & Associates, Flagstaff, Ariz.), did not alter the morphologic features of the peritoneum or result in adhesion formation.[10]

These two barriers have distinctly different survival characteristics, with oxidized regenerated cellulose being rapidly degraded enzymatically and removed by tissue phagocytes (i.e., macrophages[11]) while expanded polytetrafluoroethylene remains intact indefinitely.[12] The extent to which biomaterials incite an inflammatory response in tissue has primarily been assessed by implanting the material in a solid organ, such as muscle, and noting the presence of leukocytes in the surrounding area.[13, 14] This is not comparable to the clinical use of these surgical barriers where the material is placed over surgically traumatized peritoneum in contact with the peritoneal fluid with its unique complement of immune cells. When used in an animal model more closely resembling clinical use, oxidized regenerated cellulose, but not expanded polytetrafluoroethylene, unexpectedly caused a localized peritoneal injury and de novo

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adhesions,[10] illustrating the problem with the traditional approach to testing of biomaterials.

Although the mechanism of the tissue destruction caused by oxidized regenerated cellulose has not been established, we hypothesize that because of its biodegradability it represents a greater inflammatory stimulus than does expanded polytetrafluoroethylene. To test this hypothesis, we evaluated the effect of intraperitoneal oxidized regenerated cellulose and expanded polytetrafluoroethylene on the cellular constituents of the peritoneal fluid in a mouse paradigm used extensively to study peritoneal wound healing.[15, 16] In addition to determining the numbers of peritoneal fluid leukocytes elicited by each material, we assessed features associated with activation of the peritoneal fluid macrophages, including morphologic features,[17] the rate of adherence to plastic,[18] and stimulated hydrogen peroxide production.[19, 20] The results demonstrate that oxidized regenerated cellulose, to a greater extent than expanded polytetrafluoroethylene, elicits an acute peritoneal fluid inflammatory exudate composed of increased numbers of activated macrophages.

Material and methods

Animals, materials, and media. The animals used in these experiments were 6-week-old, virus-free, female mice weighing 22 to 24 gm. Unless otherwise specified, the animals were CD-1 outbred mice from Charles River Labs (Raleigh, N.C.). Some experiments required larger numbers of peritoneal fluid leukocytes because of losses inherent with Ficoll separation and other logistic considerations. In these situations, syngeneic mice, either CH3/HEJ (Jackson Labs, Bar Harbor, Me.) or C3H/HEN (National Cancer Institute, Bethesda, Md.) animals were used so that cells from several animals could be pooled with minimal risk that minor differences in histocompatibility antigens might activate the cells. The animal care and experiments were approved by the Duke University Institutional Animal Care and Use Committee.

Unless otherwise specified, the medium used in these experiments was Dulbecco's modified Eagle's medium (Hyclone Labs, Ogden, Ut.) buffered with HEPES (Sigma Chemical Co., St. Louis). All reagents were either screened endotoxin free as purchased or were heated to inactivate any residual endotoxin. The serum in the long-term incubations was heat-inactivated fetal bovine serum (Hyclone). Thioglycollate medium (Difco Laboratories, Detroit) was used to elicit peritoneal macrophages. The Ficoll–sodium diatrizoate (specific gravity 1.077, Hystopaque-1077) was obtained from Sigma. Endotoxin-screened, deionized, distilled water (GIBCO, Grand Island, N.Y.) was used for reagent preparation, and to minimize any effect on cell acherence, the phosphate-buffered saline solution used was calcium and magnesium free. The two commercially available surgical barriers, oxidized regenerated cellulose (Interceed) and expanded polytetrafluoroethylene (Gore-Tex Surgical Membrane), were kindly provided by their manufacturers.

Surgical technique. Anesthesia was induced by intraperitoneal injection of 0.2 ml of a 1:1 mixture of ketamine hydrochloride (Ketaset, Aveco Inc., Fort Dodge, Iowa), 5 mg/ml, and xylazine hydrochloride (Rompun, Haver, Mobay Corp., Shawnee, Kan.), 5 mg/ml. The skin was cleansed with 70% ethanol and the surgery was performed under clean but not sterile conditions. The suture used in all cases was 7-0 monofilament Prolene with a BV-1 taper needle (No. 8701, Ethicon, Somerville, N.J.). No animals appeared ill or died in the postoperative interval.

To minimize the effect of surgical trauma on the peritoneum, a midline dorsal skin incision was made in the lumbar area and the skin retracted laterally. A small flank incision was made into the peritoneal cavity and the barrier material inserted and positioned on the lateroventral surface of the peritoneal cavity. Sham-operated control animals simply had the surgical instruments inserted to mimic placement of a surgical barrier.

Peritoneal fluid and barrier collection. The animals were killed by decapitation to minimize traumatic bleeding into the abdominal cavity. Peritoneal fluid was collected by injecting 4 ml of Dulbecco's modified Eagle's medium with added heparin (10 U/ml) into the peritoneal cavity, and the abdomen was gently massaged to disperse the medium. The abdomen was then carefully entered through a ventral abdominal incision at the costal margin, taking care not to spill the peritoneal contents. The medium was aspirated through a pipette with a bulb tip. Another 3 ml of media was instilled, the aspiration process repeated, and the media from each animal were pooled and placed on ice.

Leukocyte differential counts and macrophage identification. Peritoneal fluid differential leukocyte counts were performed on samples prepared with a cytocentrifuge (Shandon Cytospin II, Shandon Southern Products, Ltd., Cheshire, England). The slides were air dried and stained with modified Wright's stain (Sigma Chemical Company). The peritoneal fluid leukocyte counts were determined by counting the cells in a standard clinical hemocytometer. There were five control animals, seven with oxidized regenerated cellulose and seven with expanded polytetrafluoroethylene inserted.

Flow cytometry. Animals had a circular disk (1.6 cm in diameter or approximately 2 cm^2) of either oxidized regenerated cellulose or expanded polytetrafluoroethylene inserted; they were killed 3 days later. The cells were harvested as described and centrifuged gently (50*g* for 20 minutes) at 4° C, the media were aspirated, and the cells were resuspended and washed

twice in phosphate-buffered saline solution. After resuspension in phosphate-buffered saline solution and viability determination by trypan blue exclusion, the suspension was adjusted to a final concentration of 1×10^6 viable cells per milliliter. Aliquots of cells were evaluated by flow cytometry (FACScan, Becton-Dickinson, Rutherford, N.J.). The data were plotted as contour maps for illustrative purposes. The vertical axis represents increasing granularity, whereas the horizontal axis represents increasing cell size of the peritoneal fluid cell population.

Cells from individual CD-1 animals for each of the treatment groups and pooled cells from syngeneic animals with samples from each treatment group were examined in the flow cytometer before and after Ficoll separation. Cells from syngeneic animals were collected and pooled from four control animals, three with oxidized regenerated cellulose and two with expanded polytetrafluoroethylene inserted. The results with the individual CD-1 mice and the syngeneic animals were similar.

Adherence assay. The cells for these studies were obtained in a similar fashion to those for flow cytometry. The cells were centrifuged gently (50*g* for 20 minutes) at 4° C, the media aspirated, the cells resuspended, and the centrifugation repeated. Medium was added to adjust the cell suspension to 3×10^6 viable cells per milliliter, and 0.1 ml of the cell suspension was plated over 0.1 ml of Dulbecco's modified Eagle's medium in the wells of 96-well microtiter plates (Falcon Plastics, Oxnard, Calif.). The plates were then placed in an incubator at 37° C in a humidified atmosphere of 5% carbon dioxide in air from 5 to 60 minutes. At the appropriate times, the wells were washed vigorously with Dulbecco's modified Eagle's medium twice, and 0.2 ml of fresh medium was placed over the remaining adherent cells and the plate returned to the incubator. After the last time point had been reached (60 minutes), a protein assay was performed by the method of Lowry et al. on each well. There were three separate experiments performed with individual mice used for each treatment; the assays were performed in triplicate. Thioglycollate, a known activator of macrophages, was used as a positive control.

To compensate for possible individual animal variation, the experiments were repeated twice with pooled cells from syngeneic animals. The pooled cells were subjected to Ficoll separation to eliminate any contamination with granulocytes. These experiments were performed with 15 control animals, nine with oxidized regenerated cellulose inserted, eight with expanded polytetrafluoroethylene inserted, and three with thioglycollate injected. The assay was again performed in triplicate and the results were similar to those performed with the outbred mice.

Hydrogen peroxide assay. Because of the need to separate granulocytes, which also produce hydrogen peroxide, a Ficoll separation was performed on pooled cells from syngeneic mice. The cells were washed twice with Dulbecco's modified Eagle's medium, resuspended, layered over Ficoll, and centrifuged for 30 minutes at 1200 to 1500 revolutions/min to separate the granulocytes. The interface was harvested and washed twice in Dulbecco's modified Eagle's medium, the viable cells were counted, and the final cell concentration was adjusted to 4×10^6 viable cells per milliliter. Aliquots of 0.1 ml were placed in wells of 96-well microtiter plates. The cells were allowed to adhere for 1 hour in an incubator at 37° C in humidified atmosphere of 5% carbon dioxide in air and washed twice vigorously with phosphate-buffered saline solution to remove nonadherent cells; the hydrogen peroxide assay was performed as previously reported.[21] The amount of hydrogen peroxide produced with and without stimulation by 200 nmol/L phorbol 12,13-myristate acetate was measured as a reflection of optical density in the horseradish peroxidase–phenol red assay. This dose of 12,13-myristate acetate was previously observed to maximally stimulate hydrogen peroxide production in human monocytes. Again, thioglycollate was used as a positive control. The experimental groups were run in triplicate. Three separate experiments were performed with a total of 22 control mice, 13 with oxidized regenerated cellulose inserted, 12 with expanded polytetrafluoroethylene inserted, and five in which thioglycollate was injected in the peritoneal cavity.

Scanning electron microscopy. The retrieved membrane specimens were briskly rinsed in 0.1 mol/L cacodylate and 5% sucrose buffer at pH 7.4 and fixed in 3% gluteraldehyde in this buffer at 4° C overnight. The specimens were then rinsed two times in the buffer for 5 minutes and dehydrated in a standard sequence, critical point dried, and sputter coated with gold-palladium. These preparations were examined and photographed in a scanning electron microscope (JEOL JSM 35C, JEOL, Tokyo). Three separate animals had each membrane inserted, and the scanning electron microscopic appearance was consistent within the two groups.

Statistical analysis. The descriptive statistics used in the figures are means and either standard deviations or standard errors of the mean as indicated. Analysis of variance was used to detect the presence of significant differences and treatment contrasts (least significant difference) used to detect differences at individual data points. For all the statistical analyses, the level of significance was considered to be $p < 0.05$.

Results

Peritoneal cell population. All the cells present in the peritoneal fluid were morphologically identifiable as leukocytes, and the differential counts are depicted in Fig. 1. The relative proportions of the various types

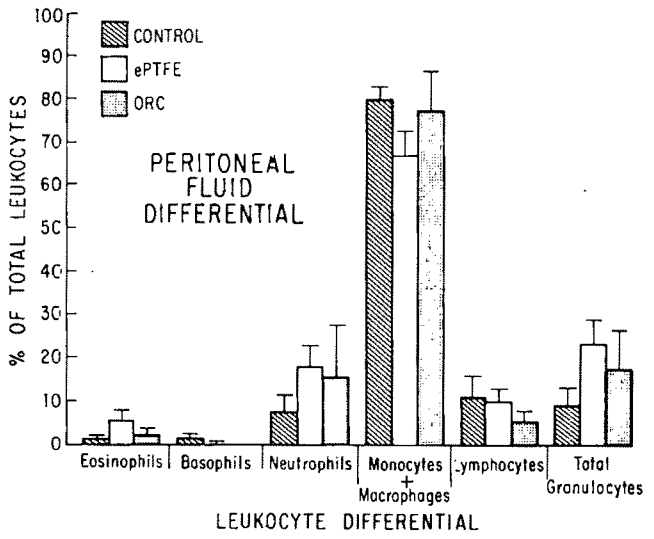


Fig. 1. Peritoneal fluid cell differential counts. Cytospin differential counts are presented. Note that they appear to be comparable regardless of material inserted except for suggestion of difference with slight increase in percentage of granulocytes when expanded polytetrafluoroethylene *(ePTFE)* was inserted. *ORC*, Oxidized regenerated cellulose.

of leukocytes were comparable to those of the control animals, except that there was a slightly greater number of granulocytes, primarily neutrophils, in the animals with expanded polytetrafluoroethylene inserted.

The mononuclear cells in the control and expanded polytetrafluoroethylene mice had the typical appearance of resident peritoneal fluid macrophages (Fig. 2, *A* and *B*). They are larger than lymphocytes with an irregularly shaped nucleus and a relatively small amount of cytoplasmic vacuolization. By contrast, when oxidized regenerated cellulose was inserted the macrophages were larger with very prominent vacuolization and marked ruffling of their plasma membranes, characteristic of activated phagocytes[17] (Fig. 2, *C*). Cytoplasmic debris consistent with ingested oxidized regenerated cellulose was frequently observed.

The total number of peritoneal fluid leukocytes in all the groups is depicted in Fig. 3 as a function of time from insertion of the material. In the control animals there was a gradual increase in the number of resident peritoneal leukocytes over the 4-week period of observation. The presence of oxidized regenerated cellulose was associated with a rapid and marked increase in the number of leukocytes, reaching maximal levels within the first 24 hours after the insertion of the material. This was followed by a gradual return to control levels over the next several days, being indistinguishable from the control animals by 5 days. The animals with expanded polytetrafluoroethylene also experienced an increase in cell number, but more slowly than with the oxidized regenerated cellulose, with a small increase in number of leukocytes on day 3 but a value comparable to controls thereafter.

Flow cytometry. The cytomorphometric patterns of the peritoneal fluid cell populations in the various groups are presented in the contour maps shown in (Fig. 4). In the control animals, the three distinct cell populations, lymphocytes, monocytes-macrophages, and granulocytes were observed. Ficoll separations were used to confirm the identity of granulocytes on the contour maps, as only mononuclear cells (monocytes-macrophages and lymphocytes) remain at the interface. Differential counts of the samples before and after Ficoll separation confirmed the reduction in granulocytes on the contour maps from 8% ± 2% to 2% ± 1%. The monocytes and macrophages were intermediate in granularity but are larger than lymphocytes. The newly recruited monocytes were not distinguishable from resident macrophages, although the former are typically smaller with activation causing macrophages to enlarge.

The insertion of expanded polytetrafluoroethylene resulted in an increase in the number of granulocytes present. The granulocytes were the most identifiably changed in this group, confirming the differential counts. The insertion of oxidized regenerated cellulose resulted in a dramatic shift toward larger macrophages with more granularity, consistent with their cytospin appearance. Oxidized regenerated cellulose produced no change in the granulocyte population from the controls. There was no apparent change in the lymphocyte populations in any of the experimental groups.

Hydrogen peroxide assay. The results of the hydrogen peroxide assays are shown in Fig. 5. Basal hydrogen peroxide production by peritoneal fluid macrophages was not detectable in any group except the

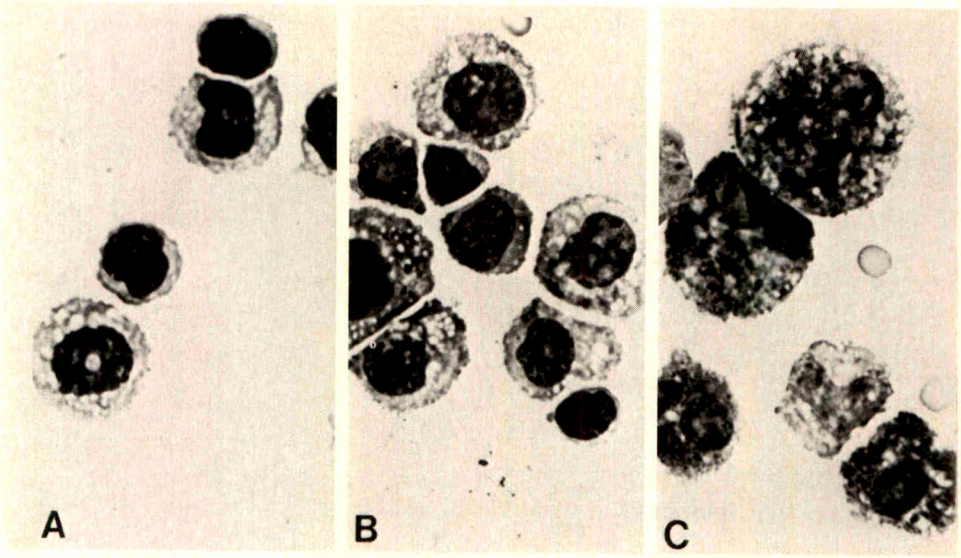


Fig. 2. Photomicrographs of peritoneal fluid macrophages. Peritoneal fluid macrophages from representative control mouse shown in **(A)** and those from animal with expanded polytetrafluoroethylene inserted **(B)** appear comparable. By contrast, macrophages from animal with oxidized regenerated cellulose inserted **(C)** were larger and had irregular plasma membranes and more granular cytoplasm. These photomicrographs were taken from cytospin preparations stained with Wright's stain. (Original magnification ×1000.)

positive thioglycolate control. 12,13-Myristate acetate–stimulated hydrogen peroxide production was noted in all the groups with the control animals' cells being significantly lower than the oxidized regenerated cellulose, expanded polytetrafluoroethylene, and thioglycollate groups. All the stimuli used increased peritoneal fluid leukocyte number and also increased the 12,13-myristate acetate–stimulated hydrogen peroxide production, with only the thioglycollate being sufficiently stimulatory to increase the basal hydrogen peroxide production.

Adherence assay. The rate of adherence for the various groups is presented in Fig. 6. The adherence was measured as cell protein remaining in the wells after vigorous washing and was plotted as a function of time. The rate of adherence was most rapid with the thioglycollate and oxidized regenerated cellulose groups and lower with the macrophages from the control and expanded polytetrafluoroethylene animals. As the cell protein might be influenced by the larger size of the macrophages in the one oxidized regenerated cellulose group, the data were also plotted as the percentage of the maximal adherence at 60 minutes. The data were essentially unchanged, indicating that the peritoneal fluid cells from oxidized regenerated cellulose animals clearly have an increased rate of adherence that is comparable with that of the positive control, thioglycollate.

Appearance of the surgical barriers

Gross findings. The expanded polytetrafluoroethylene remained intact, unchanged, and free-floating in all animals throughout the observation interval. By contrast, the oxidized regenerated cellulose was intact and relatively unchanged 6 hours after insertion but by 24 hours had become relatively gelatinous. By 3 days, the oxidized regenerated cellulose had been degraded to the point that, in most animals, it could not be found except for occasional fragments scattered throughout the peritoneal cavity. No mice in the expanded polytetrafluoroethylene group had adhesions, whereas mice in the oxidized regenerated cellulose group had adhesions with approximately the same frequency as that noted previously (58%).[10]

Scanning electron microscopy. The expanded polytetrafluoroethylene had two cell populations when present at 6 hours, rounded cells with microplicae characteristic of macrophages (confirmed by phagocytosis of polystyrene latex beads and transmission electron microscopy) and flat polygonal cells representing mesothelial cells (Fig. 7). Both cell populations were increased at 24 hours, but the cell density remained relatively light with little cell-cell contact. By 7 days the mesothelial cells virtually covered the expanded polytetrafluoroethylene with only occasional macrophages on the surface. The expanded polytetrafluoroethylene remained free-floating in the peritoneal cavity with the attached cells making no other significant cell contacts.

The oxidized regenerated cellulose had predominantly macrophages adherent at 6 hours, with the numbers of cells increasing rapidly by 24 hours when mesothelial cells first appeared in significant numbers (Fig. 7). In contrast to the expanded polytetrafluoroethylene, the oxidized regenerated cellulose was virtually

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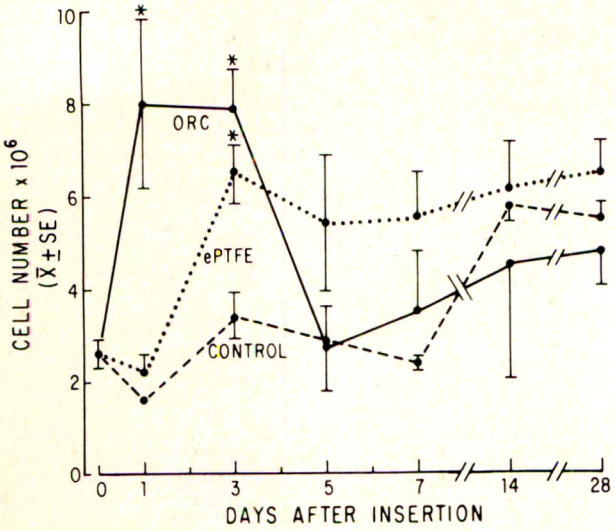


Fig. 3. Peritoneal fluid leukocyte number. Number of peritoneal fluid leukocytes for all three groups is depicted up to 28 days. Number of mice in each group varied as peritoneal fluid cells were used simultaneously in other experiments. Average number of animals for each data point was six. Peritoneal fluid cell numbers for oxidized regenerated cellulose are significantly different from those of controls on days 1 and 3 *(asterisks)*, whereas numbers for expanded polytetrafluoroethylene are different only on day 3. There is a gradual increase in peritoneal fluid leukocyte counts of control animals over interval of observation.

encased by macrophages and mesothelial cells at 24 hours, consistent with an attempt to isolate the degrading oxidized regenerated cellulose. The surface appearance of the cells on the oxidized regenerated cellulose is dramatically different from that of those on the expanded polytetrafluoroethylene, with the cells having very ruffled plasma membranes and extensive intercellular attachments, features suggesting activation. The fragments of oxidized regenerated cellulose remaining at 3 days had an appearance comparable to that of the 24-hour samples.

Comment

Oxidized regenerated cellulose has been viewed as relatively nonreactive, on the basis of traditional biomaterials testing methods, yet it clearly has a destructive effect on normal murine peritoneum.[10] Several factors may contribute to the inability of the solid tissue model to predict the impact of oxidized regenerated cellulose on the peritoneum. First, using histologic examination to determine the extent of the inflammatory exudate surrounding an implant in solid tissue is not an accurate method to assess the number, character, and state of activation of adjacent leukocytes. Second, features other than the chemical composition of the material,

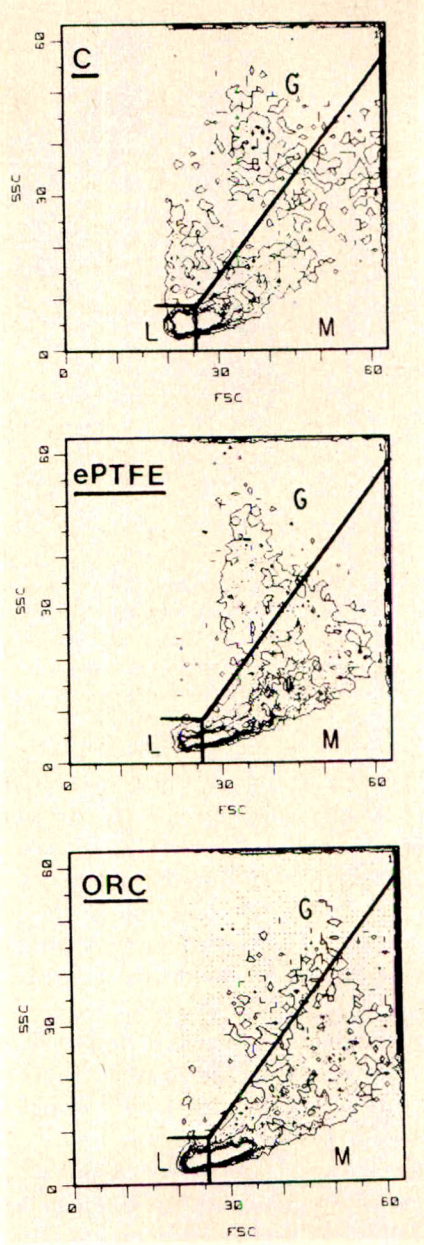


Fig. 4. Flow cytometry contour maps of peritoneal fluid cells. Representative experiment with pooled cells from syngeneic animals is presented. *Vertical axis* represents increasing granularity, whereas *horizontal axis* represents increasing cell size of peritoneal fluid cell population. Areas for individual cell types on basis of differential counts before and after Ficoll separations are marked with *L* for lymphocytes, *G* for granulocytes, and an *M* for monocytes-macrophages. Note all groups had comparable populations of lymphocytes and control animals had scattered distribution of granulocytes and macrophages. The most striking aspect of peritoneal fluid cells of expanded polytetrafluoroethylene *(ePTFE)* animals was an increase in granulocytes, whereas oxidized regenerated cellulose *(ORC)* animals had few granulocytes and much greater diversity in size and granularity in monocyte-macrophage population.

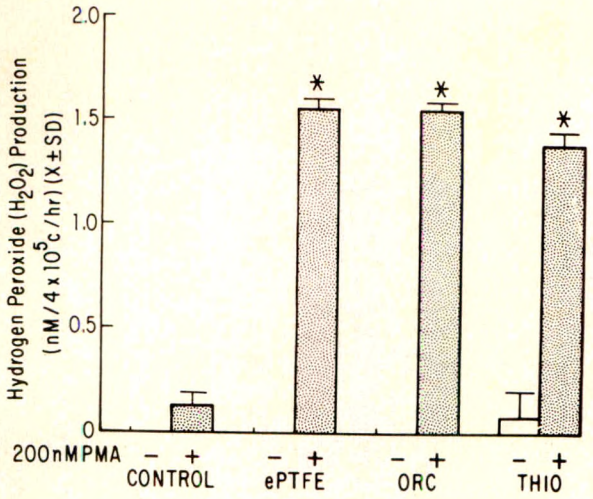


Fig. 5. Hydrogen peroxide secretion by peritoneal macrophages. Basal and 12,13-myristate acetate *(PMA)*–stimulated production of hydrogen peroxide by various groups in a representative experiment is depicted. 12,13-Myristate acetate–stimulated hydrogen peroxide production was significantly increased for all experimental groups *(asterisks)*, whereas only the thioglycollate *(THIO)* group (positive control) had detectable basal hydrogen peroxide production. *ePTFE*, Expanded polytetrafluoroethylene; *ORC*, oxidized regenerated cellulose.

such as its shape or rigidity, may alter the leukocytic response in solid tissue.[22] Last, and most important, implanting the material in a homogeneous solid organ may not reflect the clinical use of the material, as the response may be site specific. This is particularly true when tissue such as peritoneum adjacent to the barrier is not designed to withstand even minor insults and is in contact with peritoneal fluid and its unique complement of immunologically active cells. Thus, as appears to be the case with oxidized regenerated cellulose, a material thought to be nonreactive by traditional biomaterials testing may have unanticipated effects at the site of its clinical use.[10]

The ideal surgical barrier for adhesion prevention would be composed of material that is rapidly biodegradable yet nonreactive. These characteristics are not likely to be simultaneously achievable, as virtually any material that undergoes rapid degradation does so by eliciting a localized inflammatory response. Monocytes are attracted to the site of a foreign material as a result of chemotactic signals integral to the inflammatory process and, most germaine to this situation, dead and dying cells.[17] Once recruited, monocytes undergo differentiation into tissue macrophages[23] and can be activated by a variety of effectors, most prominently by the membrane perturbation accompanying phagocytosis.[17] Activated macrophages can be distinguished morphologically from resident macrophages by their increased size, granularity, and the ruffled appearance of the plasma membrane.[17] There are numerous functional changes resulting from activation, including increased biosynthetic and metabolic activity, more rapid attachment to surfaces,[18] and an enhanced capacity to generate reactive oxygen metabolites.[19, 20] Although these changes may enhance the primary function of macrophages, antimicrobial activity, the simultaneous secretion of proteinases degrades the surrounding extracellular matrix and extends the tissue injury.[17] Furthermore, a secretory product of activated macrophages, interleukin-1, stimulates fibroblast proliferation and collagen synthesis,[24] increasing the potential for adhesions to form during the healing process. Thus, if a barrier elicits an inflammatory exudate, it may further damage the surgically traumatized surface it is trying to isolate and inadvertently increase the likelihood of forming the adhesions it is trying to prevent.

The peritoneal fluid leukocytes elicited by oxidized regenerated cellulose appear distinctly different from those in the control and expanded polytetrafluoroethylene animals. Their morphologic features, coupled with the faster adherence to plastic and marked 12,13-myristate acetate–stimulated hydrogen peroxide production, suggests they are activated. The oxidized regenerated cellulose–induced peritoneal fluid leukocytosis dissipated in several days, coincident with the disappearance of the material and the healing of the peritoneum. This pattern is most consistent with an acute injurious effect of the material and the simultaneous elicitation of an acute inflammatory exudate that dissipates as the material is degraded and the peritoneum recovers from the insult. The direct effect of the oxidized regenerated cellulose on the peritoneum independent of the influence of the elicited leukocytes cannot be addressed in this paradigm. The peritoneal fluid phlogistic response associated with oxidized regenerated cellulose may be a normal component of wound healing after an oxidized regenerated cellulose–induced injury or part of an intense localized inflammatory response elicited to degrade the oxidized regenerated cellulose, consistent with the electron microscopic appearance. Biomaterials have inherent differences in the degree to which they represent inflammatory stimuli, and oxidized regenerated cellulose may have a unique propensity to elicit leukocytes.

Nondegradable materials have been selected for use in surgical devices when they appear to represent a minimal inflammatory stimulus, on the basis of the solid tissue model. These materials may nonetheless elicit a peritoneal fluid leukocytic response.[25] Because expanded polytetrafluoroethylene is not associated with

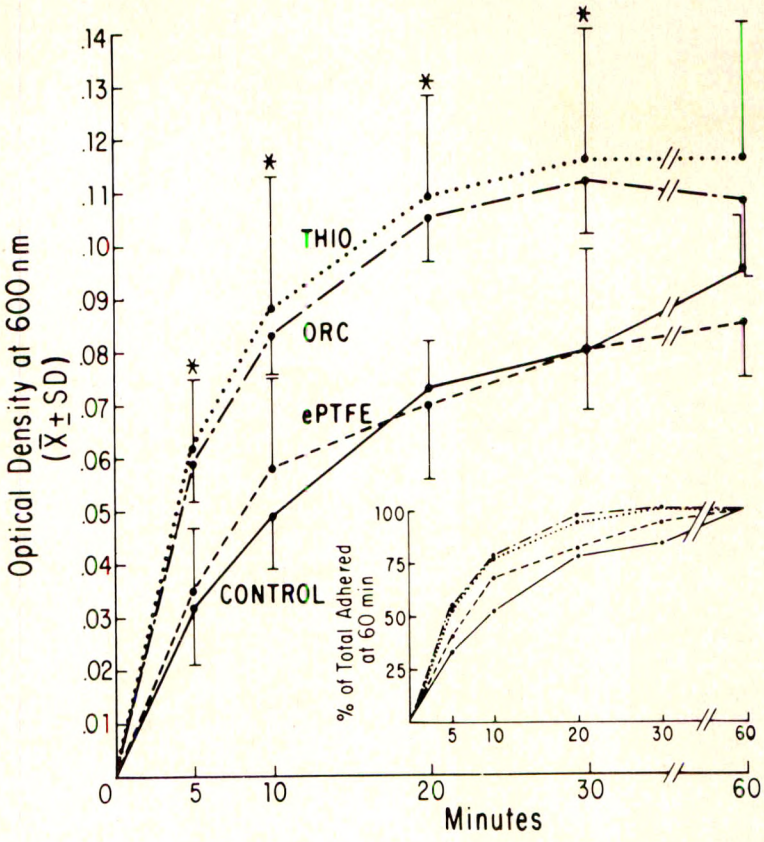


Fig. 6. Peritoneal macrophage adherence assay. Representative experiment with pooled cells from syngeneic animals after Ficoll separation is presented. Rate of adherence of peritoneal fluid cells, as indicated by cell protein in wells, is plotted versus time. Cells were maximally adherent by 60 minutes with total protein somewhat higher in oxidized regenerated cellulose and thioglycollate (positive control) groups. Note that time points on curve for oxidized regenerated cellulose that were significantly different from controls were 5, 10, 20, and 30 minutes *(asterisks)*. No time points on the curve for expanded polytetrafluoroethylene were different from controls. In *inset*, data are replotted as percentage of total protein remaining at 60 minutes, to compensate for possible differences in protein content between groups as a result of increased size after stimulation. Increased rate of adherence of oxidized regenerated cellulose–elicited cells remains.

tissue injury in spite of a mild increase in the number of normal-appearing peritoneal fluid leukocytes, other factors such as the level of activation of the elicited macrophages may be critical.

The pattern of peritoneal fluid leukocytes when expanded polytetrafluoroethylene is inserted is remarkably different from that with oxidized regenerated cellulose, with the morphologic features of the macrophages comparable to those of controls and a more gradual and smaller increase in the peritoneal fluid leukocyte count. Although the expanded polytetrafluoroethylene–induced peritoneal fluid cells could be stimulated to produce hydrogen peroxide comparable to that of the oxidized regenerated cellulose–treated animals, the adherence of the expanded polytetrafluoroethylene–induced cells was not increased over that of controls. These findings suggest that expanded polytetrafluoroethylene generates a minimal peritoneal fluid reaction in spite of the scanning electron microscopy demonstration of intimate cell-material contact. This is consistent with the extensive clinical experience with expanded polytetrafluoroethylene used as a permanent replacement for pericardium to prevent cardiac adhesions.[12]

Species differences are always a concern when data are extrapolated from animal models to humans. The murine model has long been used by immunologists and has contributed substantially to our understanding of the human immune system. In particular, the murine model has proved useful for study of the activation of peritoneal fluid mononuclear phagocytes. In spite of size differences between species, it is unlikely that the

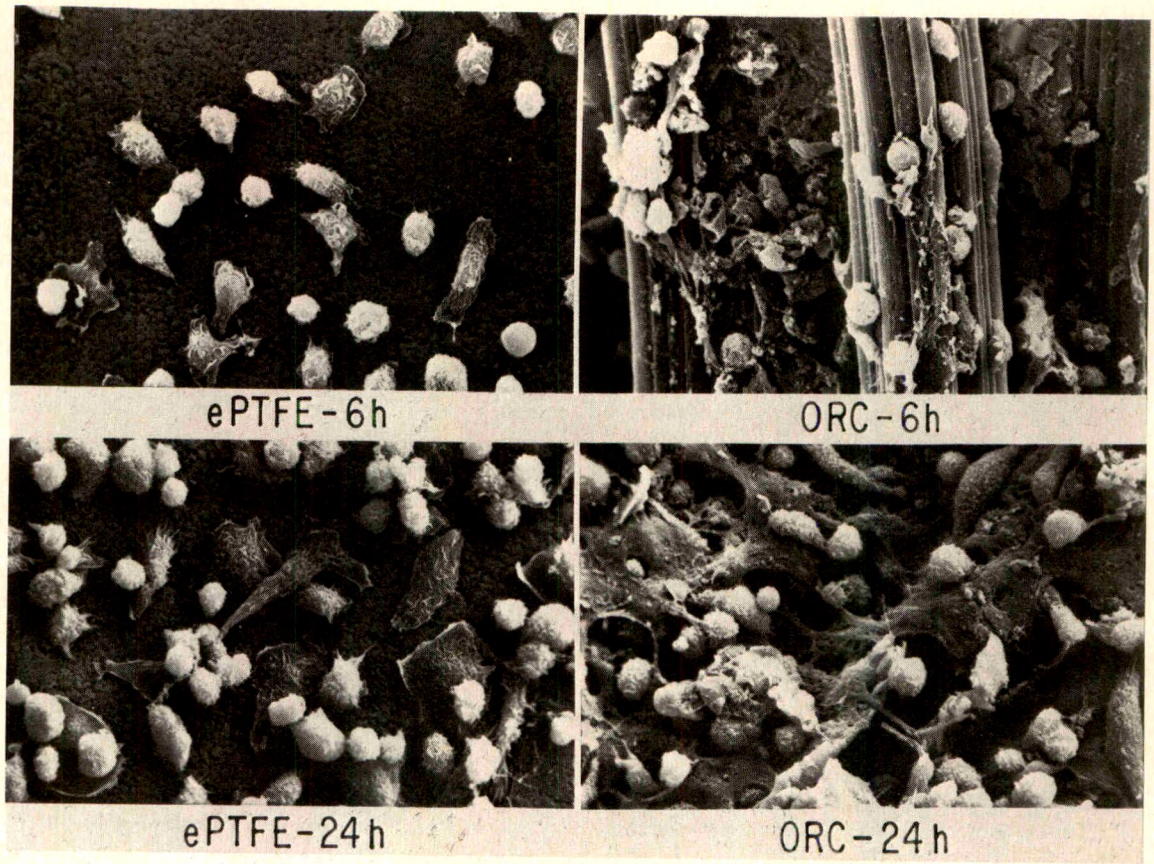


Fig. 7. Scanning electron microscopy of expanded polytetrafluoroethylene *(ePTFE)* and oxidized regenerated cellulose *(ORC)* barriers. Scanning electron micrographs of expanded polytetrafluoroethylene and oxidized regenerated cellulose barriers are presented at 6 and 24 hours after insertion. (Original magnification ×1000.) Note characteristic rounded shape of macrophages and flat polygonal mesothelial cells best demonstrated on expanded polytetrafluoroethylene. Although oxidized regenerated cellulose was visible at 6 hours, it was virtually encased by metabolically active–appearing cell investment by 24 hours (see description in Results).

basic peritoneal immunologic response or the local peritoneal impact of a biomaterial would be substantially different between the mouse and the human.

In summary, the acute peritoneal fluid exudate associated with the insertion of oxidized regenerated cellulose, as well as the tissue destruction and adhesion formation previously observed,[10] suggests that, as currently constructed, oxidized regenerated cellulose may not be an ideal material for use as a surgical barrier to prevent peritoneal adhesions. The fact that expanded polytetrafluoroethylene was not associated with a peritoneal fluid inflammatory response comparable to that with oxidized regenerated cellulose indicates that it represents less of a peritoneal fluid inflammatory stimulus. When the clinical application of a biomaterial is on the peritoneal surface, it may be important to consider the peritoneal fluid leukocyte response, as this may be a more sensitive marker of tissue reactivity than the leukocytic reaction surrounding a biomaterial inserted into solid tissue.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Miles Novy, Bearverton, Oregon (Fellow). The etiology of pelvic adhesions includes infection and other types of peritoneal irritation, but surgical trauma is the most important contributing factor.[1] The formation of postoperative adhesions is a major factor in limiting the success rate of reconstructive pelvic surgery for infertility. The mechanism of adhesion formation is a variation of the normal healing process. Injury to the peritoneum increases the permeability of blood vessels, which results in a proteinaceous exudate and coagulum within hours after the injury. Within 12 to 24 hours after injury, the fibrinous coagulum is infiltrated by numerous leukocytes and macrophages. Spontaneous dissolution of the coagulum occurs within a few days by the development of tissue fibrinolytic activity. Mesothelial regeneration of the peritoneal defect is completed by day 8 through metaplasia of underlying fibroblasts or primitive mesenchymal cells. Abnormal peritoneal healing occurs when fibrinolytic activity is inhibited (as by ischemia) and there is persistence of intraperitoneal fibrin. Ingrowth of fibroblasts and vascularization results in permanent adhesion formation.

A variety of methods have been used to prevent the development of adhesions after pelvic surgery, but meticulous surgical technique remains the cornerstone of adhesion prevention. The history of infertility surgery is punctuated by cycles of premature enthusiasm followed by disillusionment with one adjuvant agent after another. We witnessed the widespread use of dexamethasone and promethazine in the 1960s and 1970s. This was followed by enthusiasm for intraabdominal instillation of 32% dextran 70 to provide a flotation bath for abdominal viscera and traumatized peritoneal surfaces. More recently, we have seen the introduction to clinical use of mechanical barriers, notably Interceed, an oxidized regenerated cellulose, and Goretex, a polytetrafluoroethylene fabric, to separate adjoining surfaces during the healing process. As has been true with most other adjuvants for adhesion prevention, Interceed was introduced with a hypothetic rationale for its mechanism of action and limited background work in animal models. A single, prospective, randomized, multicenter clinical trial showed a reduction in peritoneal sidewall adhesion reformation,[2] but the abstruse scoring system used and the limitation to a single surgical site leave room for doubt about its real clinical efficacy. Goretex has found clinical use as a pericardial substitute. Clinical studies are under way but it has not yet been commercially exploited for infertility surgery. Interceed is closely related to oxidized regenerated cellulose (Surgicel, Johnson & Johnson Medical, Inc., New Brunswick, N.J.), differing only in degree of oxidation and the density of the weave pattern. Surgicel, a hemostatic agent, was not efficacious in most animal

models tested and was noted to be adhesiogenic in some.[3, 4] Many of us have been skeptical, to paraphrase an old metaphor, that you could weave a silk purse from a sow's ear. Interceed is being advertised as a nonreactive substance and is openly promoted for use in wrapping the ovaries and fallopian tubes after reconstructive surgery and for covering myomectomy incisions, indications for which there are no clinical or animal studies to document efficacy. Uncontrolled observations in my practice at second-look laparoscopy or repeat laparotomy and discussions with colleagues suggest that Interceed is potentially adhesiogenic at these different sites of application and under varying surgical and pathologic circumstances.

Dr. Haney's research article is a refreshing entry in the midst of this controversy and provides controlled observations on the consequences of applying barrier materials for peritoneal adhesion prevention in a murine animal model. In a companion article to be published in *Fertility and Sterility*,[5] Dr. Haney demonstrated that simple peritoneal apposition of oxidized regenerated cellulose (Interceed) caused localized sloughing of the mesothelial layer and de novo adhesion formation to adjacent viscera in 58% of animals. Neither sham-operated animals nor animals receiving polytetrafluoroethylene (Goretex) had development of peritoneal injury or adhesions.

The objectives of the study reported here were to explore the mechanisms of the peritoneal tissue damage and adhesion formation to the oxidized regenerated cellulose barrier and to characterize and compare the peritoneal inflammatory response with absorbable barrier (oxidized regenerated cellulose) and a nonabsorbable barrier (expanded polytetrafluoroethylene) in the mouse. Oxidized regenerated cellulose is rapidly degraded enzymatically and removed by macrophages, whereas expanded polytetrafluoroethylene remains intact indefinitely. The authors propose that peritoneal damage by oxidized regenerated cellulose is caused by the greater inflammatory response that is elicited in comparison with expanded polytetrafluoroethylene. Previous studies of the inflammatory response to barrier materials have been confined largely to soft tissues or to histologic studies at the site of injury. In the current work more specific qualitative and quantitative assays of peritoneal leukocyte function were used, i.e., differential counts and macrophage identification by flow cytometry, adherence assays, and hydrogen peroxide assays to determine macrophage activation. Finally, scanning electron microscopy demonstrated contact between cells and barrier material and to characterize the fine structure of activated macrophages.

A rapid and marked increase in total peritoneal leukocyte numbers was observed with oxidized regenerated cellulose in contrast to expanded polytetrafluoroethylene or control animals. The principal distinguishing feature was a dramatic shift to activated macrophages with oxidized regenerated cellulose. Activation was confirmed by the adherence assay and morphologic criteria, although the hydrogen peroxide assay showed no difference in capacity to generate reactive oxygen metabolites. The data presented by Dr. Haney provide only partial support for macrophage recruitment as the mechanism by which oxidized regenerated cellulose causes peritoneal damage. Macrophage activation results in numerous functional changes, principally the release of cytokines and the secretion of proteolytic enzymes; those responses were not measured in this study. Furthermore, the authors did not assess a potentially direct toxic effect of either product on peritoneum independently of the inflammatory response. Nor did they evaluate the possibility that either may have an inhibitory effect on tissue fibrinolytic activity. The murine model used in the current work differs from previous animal models where uterine horn scrapings and peritoneal sidewall defects were created and adhesion scores were compared in treated and control animals. For instance, in the rabbit studies of Diamond et al.,[6] oxidized regenerated cellulose application resulted in a 24% reduction in the area of the traumatized sidewall that was found to be involved with adhesions at the time of necropsy. Criticism may be leveled against the current study because it does not approximate the clinical situation where the barrier material is placed over a site of injury. However, Dr. Haney argues convincingly that first it is important to determine the effect of any surgical material on the normal tissues with which it will come in contact. Although it is logical, it does not necessarily follow that a barrier material that injures normal peritoneum will cause more adhesions when it is applied to surgically traumatized peritoneum.

I would like to ask Dr. Haney the following questions:

1. Do you have preliminary data or plans to assess possible direct toxic effects of oxidized regenerated cellulose on explants of peritoneum or in tissue culture? Do you have evidence for inhibition of fibrinolytic activity by either product?
2. Would you comment on any qualitative or quantitative differences between free peritoneal macrophages as studied by you and resident macrophages at the site of injury? Are the free cells representative of the adherent macrophages in terms of enzyme activity or the ability to produce free oxygen radicals?
3. Peritoneal healing is not complete until 5 to 8 days after injury and oxidized regenerated cellulose disappears within 3 days after application. Are these observations compatible with the hypothetic mechanism of action, namely, that it prevents the deposition of fibrin bands and subsequent fibroblast ingrowth?
4. How do you reconcile your observations in the mouse with those of Diamond et al.[6] in the rabbit uterine horn and sidewall defect model? Is this a difference in the methods used to develop adhesions, in the site of application, or in adhesion scoring?

5. On the basis of your experiments, you have stated that oxidized regenerated cellulose is not an ideal material to be used as a surgical barrier to prevent adhesions to peritoneal surfaces. In this context, could you comment on possible limitations of the multicenter clinical trial in which you were a participant? For instance, do you believe that the results of that study justify its extended use at other pelvic sites and in other operative situations?
6. Would you speculate on a possible specific application for expanded polytetrafluoroethylene or a similar material in infertility surgery? Is it possible to reconcile the inherently inflammatory aspects of a biodegradable material with the ability to prevent adhesions?

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DR. ALAN H. DE CHERNEY, New Haven, Connecticut. The questions I have, first of all, are regarding expanded polytetrafluoroethylene. Why did he not see any adhesion formation in his expanded polytetrafluoroethylene model in a previous study in regard to suture material? We usually noted that the area where we sutured the expanded polytetrafluoroethylene in place was predisposed to adhesion formation.

Also, I was surprised—and I wondered if he was as well—that this product had the inflammatory response that it did. Although it wasn't equivalent to that of the oxidized regenerated cellulose, it certainly was a marked inflammatory response. We have been led to believe that expanded polytetrafluoroethylene is relatively noninflammatory.

My next question is about mass effect. How much of the effect that you noted was due to the mass of material that was placed in the cavity? We did a study a number of years ago where we showed a marked adhesion effect by putting clips in rabbits. The company that manufactured the clips then brought those clips in and showed that if we made clips the size that we put in rabbits in humans, they would be as tall as I was, and that was the reason that we had a fair amount of adhesions. So how much of this is due to mass effect?

Last, what was his observation at the time the animals were killed as far as adhesion formation was concerned? I wonder because he chose a nonstandard model to look at adhesions. I also wonder whether adhesions were generated in this model.

DR. JOHN A. ROCK, Baltimore, Maryland. As we all know, one of the modifiers in animal models for adhesions studies is the presence of blood in the peritoneal cavity. In your model, one of the important variables that should be addressed when the incision is made and the implant is placed is the amount of blood in the peritoneal cavity. It has been well established that oxidized regenerated cellulose loses its efficacy when it becomes involved with blood clots. Incorrect application is of concern. Once it loses its flat position, it becomes a glob of cellulose. Furthermore, large areas of peritoneal trauma that can occur in reproductive surgery may modify its efficacy.

In the murine mouse model infection is always an issue. This also is a confounding variable. Were some of the experiments complicated by infection? Were the animals covered by antibiotics before the sampling and were the sham controls covered by antibiotics as well?

DR. JOSEPH HILL, Boston, Massachusetts. I have a question about the methods you used. You mentioned that with your study design you did not see an increase in T or B cells. Did you perform any functional assays? I ask because the method you used does not differentiate natural killer cells from macrophage populations, and on the basis of the illustrations you presented, it is possible that some of the cells you are calling macrophages may indeed be either lymphoblasts or natural killer cells.

DR. HANEY (Closing). Dr. Hill has a long-standing interest in T cells, whereas our interest tends to focus on macrophages. I think he's correct. We did not do differential leukocyte counts in these experiments but have done them in other experiments with thioglycollate; these cells are clearly macrophages. I did not repeat the differential counts with this study, and it certainly is a reasonable criticism that some of these cells we're encountering are not all of the monocyte series.

With regard to Dr. Rock's comments about blood, we intentionally used a flank incision that's about 4 mm long and carefully excluded any animals we encountered with significant bleeding at that site. So I do not believe the flank incision for insertion will complicate the question of bleeding because there really was not much.

Infection is a constant concern. We did not treat any animals with antibiotics, sham-operated controls or others. We lost no animals. We saw no animals with cytospins that had polymorphonuclear leukocytes in the peritoneal cavity the way you would anticipate with

infection. We had no cutaneous fistulas. The only comment I can make is that they didn't lose weight and we used no antibiotics and saw no obvious evidence of any infection.

In response to Dr. DeCherney's comments, all I can say is we did not encounter adhesions to the site of the suture. We used 7-0 Prolene sutures and triple knots. I didn't show any, but we have many examples of simply putting sutures in the peritoneal cavities of our control animals and have not had any adhesions to them. The only time we've encountered adhesions at all is in other experiments when we put the suture material higher in the peritoneal cavity on the right side where it can contact the liver. With the constant breathing and the movement of the liver, the edge of the suture will abrade the liver and create an adhesion, but down below the liver edge we've just not encountered adhesions.

Now, we were a bit surprised that the expanded polytetrafluoroethylene surgical membrane activated the cell population and altered the cell number, but I don't think that should have surprised us. It's not invisible to the immune system. Expanded polytetrafluoroethylene may have a very unique or different elicitation response. So, it was a bit of a surprise on the basis of previous information that implanting these materials in solid tissue models does not lead to much inflammation. Again, I think that's the entire reason we were performing the study on the peritoneal fluid cell populations. It's really necessary to put the biomaterial in the site in which it's going to be clinically used and exposed to the immune system in the peritoneal cavity. The reaction is likely to be very different than in solid tissue models.

The mass effect of the material tends to be a frustrating issue to deal with because obviously these are metabolically different animals. The size of the barrier is not out of line with using two or three membranes in relative proportion between the mouse and the human. So, whereas I can never directly address that because these are obviously different animals, I simply think it's a fairly minimal concern.

We did not notice any adhesions when we killed the controls or the expanded polytetrafluoroethylene–treated animals. All I can say is we just didn't encounter them in our experimental study design.

Dr. Novy had several questions. We have not yet done organ cultures taking peritoneum, putting it in tissue culture, and then placing the materials. That's certainly a good idea. We also are planning on doing short-term experiments intraoperatively. I would be extremely surprised if fibrinolytic activity is not depressed by the oxidized regenerated cellulose material because anything that's acidic or generates an inflammatory response tends to depress fibrinolysis. I just don't believe there are any data to address it at the moment and we did not do so in this study.

He also asked whether the cells that cover the injured surface differ from the cells in the peritoneal cavity. In earlier experiments we placed latex beads in the peritoneal cavity so the macrophages present would ingest them. Those are the cells attached to the surface, so realistically I think they're the same cells. The possibility does exist that those cells may be altered by attachment.

He asked about the time course of the study. The rapidity with which this material dissolves is a bit inconsistent with the length of time you would like a barrier present to prevent adhesions. It is degraded extremely rapidly. The differences between these studies and others, notably, the rabbit model, are all going to be difficult to address. You have species differences and model differences. Quite frankly, the reason we chose this model as opposed to the rabbit model is that we wanted another species to look at. We could evaluate the immunologic aspects of it in the peritoneal cavity, and I think most of these materials need evaluation in more than one species to be reasonable.

Now, we participated in a randomized, prospective clinical trial with oxidized regenerated cellulose with one side getting the material and one side not, and then we submitted our data to the company. I have significant concerns about the trial, and they stem from two features. One is the number of patients in the trial. If you considered a 50% adhesion rate and would like to reduce that to 40%, to get a reasonably powerful study, you need about 400 patients per treatment arm. Luckily, with this material, from the company's perspective, one patient is two treatment arms, a control side and a treatment side, but you still are short of what's necessary to have a powerful clinical study.

Second is the issue about the abstruse nature of the scoring system. I'm not sure at all what the area of an adhesion means. An adhesion occurs between two structures. I don't think the area is a significant issue. So I remain very concerned about the validity of that trial, and clearly this material needs to be placed at other sites, on a bleeding surface or bleeding edge of a myomectomy incision or on the ovary, to be able to verify that it's efficacious at those sites. Last is the issue of biodegradability. I remain concerned that any material that's rapidly degraded will not serve this purpose well. The rapidity with which it degrades is directly proportional to its ability to elicit an inflammatory response, and inherently, I think those are in the opposite direction of our intent. So I think we're going to be learning a lot about biomaterials in the future, and that's going to be a critical issue for barrier technology.

Maternal phenylketonuria collaborative study, obstetric aspects and outcome: The first 6 years

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OBJECTIVE: The purpose of this study was to evaluate the efficacy of a phenylalanine-restricted diet in reducing fetal morbidity associated with maternal hyperphenylalaninemia in women of childbearing age with blood phenylalanine levels >240 μmol/L (>4 mg/dl) or an unrestricted diet.
STUDY DESIGN: Two hundred thirteen pregnant women with hyperphenylalaninemia that resulted in 134 live births have been enrolled in the study. Outcome measures were subject to the χ^2 test, Fisher exact test analysis of variance, *t* test, or Wilcoxon nonparametric test for analysis.
RESULTS: Optimal fetal outcome appeared to occur when blood phenylalanine levels <600 μmol/L (<10 mg/dl) were achieved by 8 to 10 weeks' gestation and maintained throughout pregnancy (trimester averages of ≤360 μmol/L (≤6 mg/dl)). Initiation of dietary therapy during the third trimester of pregnancy appears to have little beneficial effect on the fetus.
CONCLUSIONS: Preconceptual counseling and early entrance into a prenatal care program is essential in achieving optimal fetal outcome in women with hyperphenylalaninemia. (AM J OBSTET GYNECOL 1992;166:1150-62.)

Key words: Maternal phenylketonuria, fetus, mental retardation, congenital defects, dietary therapy

Phenylketonuria is an inborn error of phenylalanine metabolism that was a major cause of mental retardation.[1, 2] However, routine newborn screening for this disorder and attendant early dietary treatment have virtually eliminated mental retardation from phenylketonuria.[3] In spite of this remarkable achievement, the effort to eliminate mental retardation related to phenylketonuria has progressed through a series of successes and disappointments. Postnatal phenylketonuria-related brain damage has primarily been eradicated; however, in the course of, and to a great extent as a result of, achieving this a previously very rare deleterious effect has emerged, namely fetal damage caused by untreated maternal hyperphenylalaninemia.[4] Thus young women with phenylketonuria in whom mental retardation was prevented by early treatment during infancy and childhood are becoming pregnant and are at high risk for birth defects in their offspring.

Although this problem was identified more than 30 years ago, many questions about the disease process and the treatment of the disorder still remain. To elucidate the problems concerning maternal phenylketonuria, the National Institute of Child Health and Human Development initiated the National Maternal Phenylketonuria Collaborative Study (1984). Subsequently Canada joined this study. Specific questions addressed by the study include: (1) What phenylalanine level during pregnancy will maintain normal fetal development? (2) Is preconceptual phenylalanine restriction necessary? and (3) Is supplementation with tyrosine and various trace elements necessary for normal pregnancy outcome?

The purpose of this article is to present a preliminary report on the obstetric findings of the first 6 years of the study.

Material and methods

Study design. A prospective, longitudinal, observational study was designed by participants in the Col-

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laborative Study to evaluate the efficacy of a phenylalanine-restricted diet in reducing fetal morbidity associated with maternal phenylketonuria. The Collaborative Study involves phenylketonuria centers in 50 states, the District of Columbia, Puerto Rico, and all of the provinces in Canada, which are regionally organized under the direction of five contributing centers. The coordinating center located at Children's Hospital of Los Angeles coordinates and maintains relationships with the contributing centers for patient identification, recruitment, outreach, education, enrollment, clinical follow-up, and collection of data, which are collated at a central data bank in Los Angeles. Quarterly evaluations of the project are completed by the coordinating center and are made available to the participating clinics.

Before the initiation of the study institutional review board approval was obtained by the individual participating centers.

Inclusion criteria. The goal of the study, as determined by sample-size calculations, is to include ≥200 hyperphenylalaninemia pregnancies with offspring. Eligibility criteria consist of women of childbearing age whose blood phenylalanine concentration on an unrestricted diet is >240 μmol/L (>4 mg/dl). The subjects must be intellectually capable of understanding and signing an informed consent and willing to comply with dietary treatment and cooperate with the collaborative study staff in providing data. In addition, these women must voluntarily become pregnant at some time during the term of the project. All pregnancy outcomes, including spontaneous and elective terminations, are contained in the study.

There are two groups of control subjects. The first control group, office controls, consists of women without hyperphenylalaninemia enlisted from the same obstetric practice used by a subject with hyperphenylalaninemia. These subjects are matched for race, maternal age, gestation, and parity. The second control group, familial controls, consists of persons selected from either the same sibship of the subject, sibship of the natural father of the offspring of the subject, or first cousin of the subject. All controls are monitored prospectively. Additional comparison groups include (1) historical data from the Lenke-Levy survey of untreated hyperphenylalaninemia pregnancies,[5] (2) earlier untreated pregnancies of the study subjects, and (3) prospective evaluation of the pregnancies of mates of men with hyperphenylalaninemia.

Potential subjects are identified through a variety of sources; clinic records, national and state registries, media public service messages and national, state, and local medical meetings. On meeting study criteria informed consents are signed by all eligible women. Dietary treatment is initiated if indicated.

Subjects enroll and begin receiving treatment at either of two stages relative to pregnancies: before conception of planned pregnancies whenever possible, or after conception on the discovery of an unplanned pregnancy.

Management protocol. The treatment plan consists of providing adequate nutrition during pregnancy, offering a diet restricted in phenylalanine to women with hyperphenylalaninemia with blood phenylalanine concentrations of ≥600 μmol/L (≥10 mg/dl), maintaining blood phenylalanine concentrations between 120 and 600 μmol/L (2 to 10 mg/dl), and supplementing with tyrosine and trace elements as medically indicated.

Patients are treated at their usual phenylketonuria treatment center or at a local medical center on a monthly basis for evaluation and dietary counseling. Weight gain, diet records, and analysis of plasma trace elements and amino acids are evaluated monthly; a weekly blood sample is collected by finger stick for phenylalanine measurement to determine whether alterations in diet therapy are necessary. During a pregnancy, each woman continues to see her private physician for prenatal care. At approximately 20 weeks' gestation ultrasonographic dating of the pregnancy is obtained. Repeat ultrasonographic examinations are performed at 28 and 34 weeks' gestation for fetal evaluation and additional dating information. After delivery all children are monitored and assessed for cognitive and behavioral development.

Study evaluation. All data are collected in a standardized format. Data on maternal and offspring outcomes include the incidence of spontaneous abortion, major complications of pregnancy and delivery, gestational age at delivery, congenital malformations such as microcephaly and heart defects, and offspring growth and development. Additionally, several potentially confounding variables are included in data collection and analysis. Among these are parental intelligence quotient, parental head circumference, socioeconomic status of the parents, overall nutritional status, and exposure to teratogens such as drugs, tobacco, and alcohol. Outcome measures are stratified according to maternal hyperphenylalaninemia classification and the time of onset of treatment.

For the purpose of this report, standard statistical methods for rates and proportions were used in data analysis because many of the end points were dichotomous variables. Simple contingency table analysis was done by χ^2 or the Fisher exact test for comparison between groups on overall proportions and frequency distributions of categoric variables. Continuous variables were analyzed with analysis of variance, the t test, or the Wilcoxon nonparametric test when appropriate. A p value of 0.05 was accepted as significant.

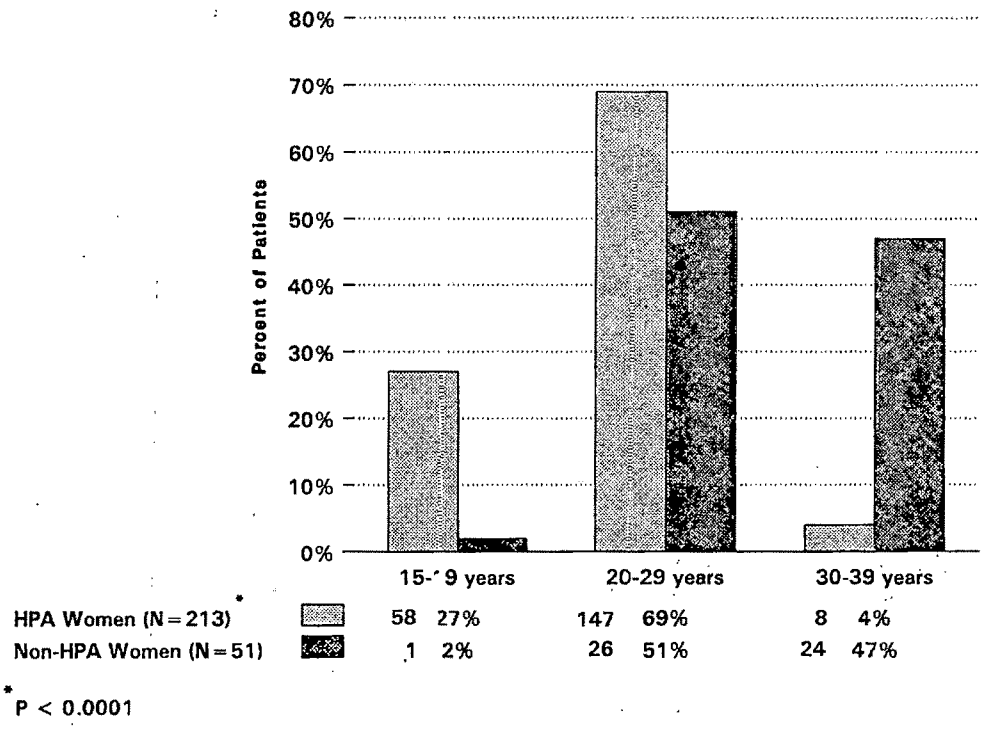


Fig. 1. Maternal age of women in the hyperphenylalaninemia *(HPA)* and control *(Non-HPA)* groups at conception.

Table I. Gestational age at enrollment for hyperphenylalaninemic and control pregnancies

	Hyperphenylalaninemia		*Control*	
	No.	*%*	*No.*	*%*
Preconception	57	27	0	0
0 to 4 Weeks	9	4	2	4
5 to 8 Weeks	59	28	12	24
9 to 13 Weeks	44	20	17	33
Second trimester	40	19	16	31
Third trimester	4	2	4	8
TOTAL	213	100	51	100

Results

A total of 285 women with hyperphenylalaninemia who had 237 pregnancies were enrolled during the first 6 years of the study. Two hundred thirteen of these pregnancies were completed, with 134 live births achieved. Concurrently, 78 pregnancies in the nonhyperphenylalaninemia control groups were entered in the study. The control group consisted of 52 office controls, 8 familial controls, 10 unaffected mothers with previous children affected by a metabolic disorder, and 8 mates of men with phenylketonuria. There were 51 completed pregnancies, all live births, among the office controls. The other groups were not included in the present data analyses because of insufficient sample size.

Comparison of hyperphenylalaninemia and control groups. The gestational age at enrollment for completed pregnancies in the hyperphenylalaninemia and control group is shown in Table I. As expected, none of the controls were entered preconceptually as opposed to 27% of the women with hyperphenylalaninemia. More control women (61%) were enrolled during their first trimester of pregnancy than women with hyperphenylalaninemia (52%).

A comparison of maternal age distribution at the time of conception of the subjects with hyperphenylalaninemia and the control subjects is shown in Fig. 1. The women with hyperphenylalaninemia were found to be significantly younger at the time of conception than the nonhyperphenylalaninemia group ($p < 0.0001$). Twenty-seven percent of the women with hyperphenylalaninemia were between 15 and 19 years old at the time of conception, whereas only 2% of the control women were a similar age when they conceived. Con-

Table II. Gestational age at time of initiation of dietary treatment of women with hyperphenylalaninemia enrolled in study

Diet status	*No.*	*%*
Hyperphenylalaninemia: Diet not offered	19	9
Phenylketonuria-Hyperphenylalaninemia: Diet initiated		
Preconception	36	17
0 to 4 Weeks	12	6
4 to 8 Weeks	60	28
8 to 13 Weeks	21	10
Trimester II	32	15
Trimester III	2	1
Phenylketonuria-Hyperphenylalaninemia: Not treated	31	14
TOTAL	213	100

Table III. Comparison of weight gain in pregnant women in hyperphenylalaninemia and control groups by weight class at conception

*Weight class**	*Weight gain (lb)*							
	Hyperphenylalaninemia				*Control*			
	n	*Mean*	*SD*	*Range*	*n*	*Mean*	*SD*	*Range*
<90	7	23	17	2 to 40	4	36	7	26 to 43
90% to 100	36	33	12	4 to 59	22	33	7	19 to 49
>110	35	30	16	−1 to 64	5	27	15	3 to 40

*Percentage of recommended weight for height.

versely, only 4% of the women with hyperphenylalaninemia were between 30 and 39 years old as opposed to 47% of the control group at conception.

Dietary therapy. As indicated in Table II, 19 (9%) of the study subjects were found to have untreated blood phenylalanine levels <600 μmol/L (<10 mg/dl) and were not offered dietary treatment. Thirty-six (17%) of the women with hyperphenylalaninemia were given phenylalanine-restricted diets before conception. Ninety-three (44%) of the women with hyperphenylalaninemia initiated diet therapy in the first trimester of pregnancy, 32 (15%) in the second trimester, and 2 (1%) in the third trimester. Thirty-one (14%) women with hyperphenylalaninemia were enrolled in the study who did not accept treatment at any time during pregnancy.

Maternal weight gain during pregnancy in women with hyperphenylalaninemia and women in the non-hyperphenylalaninemia control group can be seen in Table III. For those women who were the appropriate weight in relation to their height, mean weight gain was observed to be identical in the women with hyperphenylalaninemia and women who were unaffected. There was a tendency for women in the hyperphenylalaninemia group who were <90% of their recommended weight to gain less (23 pounds) than control women (36 pounds) in the same weight classification. Women with hyperphenylalaninemia who were >110% of their recommended weight gained slightly more weight than their control counterparts.

Obstetric history and complications. Obstetric and medical data are listed in Table IV. Significantly more women with hyperphenylalaninemia were primigravid than were controls (34%) ($p = 0.04$). No significant differences were observed in the incidence of smoking and the use of marijuana or other drugs between the groups. Women with hyperphenylalaninemia reported a significantly lower frequency of alcohol consumption than did control women ($p = 0.01$).

The incidences of diabetes, hypertension, or abnormal bleeding were not found to be significantly different between the affected and control groups.

Fetal and postnatal loss. A fetal loss rate of 37% occurred in the hyperphenylalaninemia group (Table V). Of the 79 losses observed, 37 (17% of the total pregnancies) were spontaneous abortions. The rate of spontaneous abortion among women with hyperphenylalaninemia was not different for those 5 (16%) who declined to adhere to the recommended diet compared with the 7 (19%) who began diet therapy before conception (Fig. 2). Women with hyperphenylalaninemia who had blood phenylalanine levels of <600 μmol/L (<10 mg/dl) during their pregnancies and were not offered the restrictive diet had no spontaneous abortions. When stratified by the time diet therapy was initiated the frequency of spontaneous abortion for the

Table IV. Obstetric history and medical complications of hyperphenylalaninemic and control pregnancies

	Hyperphenylalaninemia		*Control*		
	n	%	*n*	%	*p Value*
Primigravid	84	53	14	34	0.04
Smoking*	46	26	7	15	0.16
Alcohol*	30	17	17	35	0.01
Marijuana*	3	2	0	0	0.41
Drugs*	1	1	0	0	0.66
Diabetes	1	1	3	6	0.11
Hypertension	3	2	3	6	0.16
Abnormal bleeding	14	8	5	11	0.33

Percentages are based on numbers of subjects for whom data are available.
*Includes use 6 months before conception.

Table V. Comparison of fetal and neonatal loss in hyperphenylalaninemic and control pregnancies

	Trimester of termination					
	First		*Second*		*Third*	
Outcome	*HPA*	*Control*	*HPA*	*Control*	*HPA*	*Control*
Live births HPA $n = 134$ Control $n = 51$	0	0	0	1	134	50
Stillbirths HPA $n = 2$ Control $n = 0$	0	0	1	0	1	0
Spontaneous abortion HPA $n = 37$ Control $n = 0$	26	0	11	0	0	0
Elective abortion HPA $n = 39$ Control $n = 0$	26	0	12	0	1	0
Ectopic pregnancy HPA $n = 1$ Control $n = 0$	1	0	0	0	0	0

Hyperphenylalaninemia postnatal loss: three deaths from heart defects at ages 2 weeks, 2 weeks, and 22 months. Control postnatal loss: one death related to extreme prematurity. *HPA,* Hyperphenylalaninemia.

women with hyperphenylalaninemia did not significantly alter among these various classes. No fetal wastage was observed in the control group of the study.

Thirty-nine (49%) of the 79 hyperphenylalaninemia pregnancy losses were caused by elective terminations. Twenty-three (59%) of these abortions occurred in the group of women with hyperphenylalaninemia who either refused indicated dietary treatment or planned elective termination at study entry. One (1% of all losses) ectopic pregnancy, terminated at 9 weeks' gestation, occurred in a mother with hyperphenylalaninemia who initiated diet therapy at 4 weeks' gestation. In addition, 2 (3% of all losses) pregnancies resulted in stillbirths, 1 occurring at 25 weeks' gestation, 1 at 36 weeks gestation. Both women began restricted diets between 8 and 13 weeks' gestation.

The hyperphenylalaninemia group of live births resulted in 3 (2%) postnatal losses related to congenital heart defects. One (2%) neonatal loss observed in the control was associated with extreme prematurity.

Delivery outcome. A summary of the delivery outcome of the hyperphenylalaninemia and control pregnancies is listed in Table VI. No statistical difference was observed in a comparison of the hyperphenylalaninemia and control groups for the method of delivery, number of multiple births, sex, and 1- and 5-minute Apgar scores. Significant differences did occur between the two groups, however, in the percentage of infants whose birth measures of length ($p = 0.01$), weight ($p = 0.02$), and head circumference ($p = 0.0001$), were <10th percentile. None of the control pregnancies resulted in newborns with birth measurements <10th percentile; 24% of the hyperphenylalaninemia offspring had abnormally small head circumference, 11% had decreased weight, and 11% had decreased length.

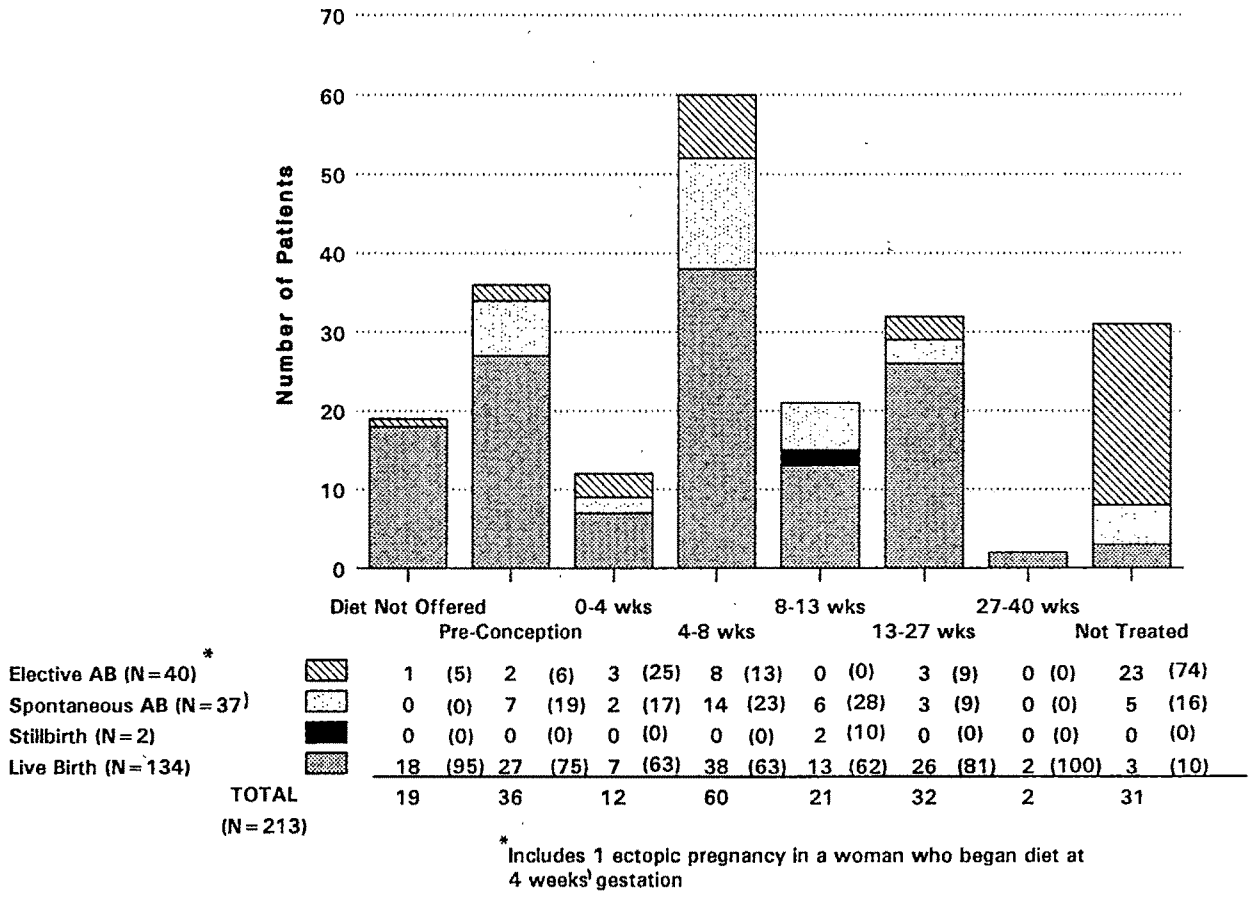


Fig. 2. Pregnancy outcome in patients with hyperphenylalaninemia stratified by gestational age at initiation of diet during pregnancy. *AB*, Abortion; ( ), percentage of total.

The dose-related effects of phenylalanine on fetal growth can be seen in Fig. 3, which illustrates the median birth measurement percentiles by gestational age at which maternal blood phenylalanine levels <600 μmol/L (<10 mg/dl) were achieved. Although notable differences in length and weight can be recognized in the offspring of those women who did not achieve dietary control until >10 weeks' gestation, head circumference was most severely affected.

Birth measures of the offspring were also analyzed according to the effects of maternal smoking on birth length, weight, and head circumference <10th percentile (Table VII). A statistically significant difference was observed only for head circumference ($p = 0.046$) in those women with hyperphenylalaninemia who had a positive history of smoking.

Congenital malformations. Congenital malformations observed at birth in the hyperphenylalaninemia population are arranged in Table VIII according to the patients' average blood phenylalanine levels for any trimester of pregnancy.

The presence of microcephaly was noted if diagnosed at birth. Ninety percent[27] of the women with hyperphenylalaninemia with phenylalanine levels of ≥1200 μmol/L (≥20 mg/dl) had microcephalic infants. Those women who had levels between 600 and <1200 μmol/L (10.1 to 19.9 mg/dl) had a 49% (19 infants) incidence of microcephaly. None of the infants born to women with hyperphenylalaninemia whose levels were ≤600 μmol/L (≤10 mg/dl) throughout pregnancy had microcephalia.

Intrauterine growth retardation is defined here as birth weight, length, *and* head circumference below the third percentile. Similar to the observations made with microcephaly, 27 (90%) of the affected subjects with phenylalanine levels of ≥1200 μmol/L (≥20 mg/dl) were found to have intrauterine growth retardation. A decrease in the incidence of the disorder was observed with a decrease in blood levels. Four (10%) of women with hyperphenylalaninemia with intermediate blood levels had intrauterine growth retardation infants. None of the offspring of the women with levels of ≤600 μmol/L (≤10 mg/dl) were abnormally small.

Six congenital heart defects were identified only in

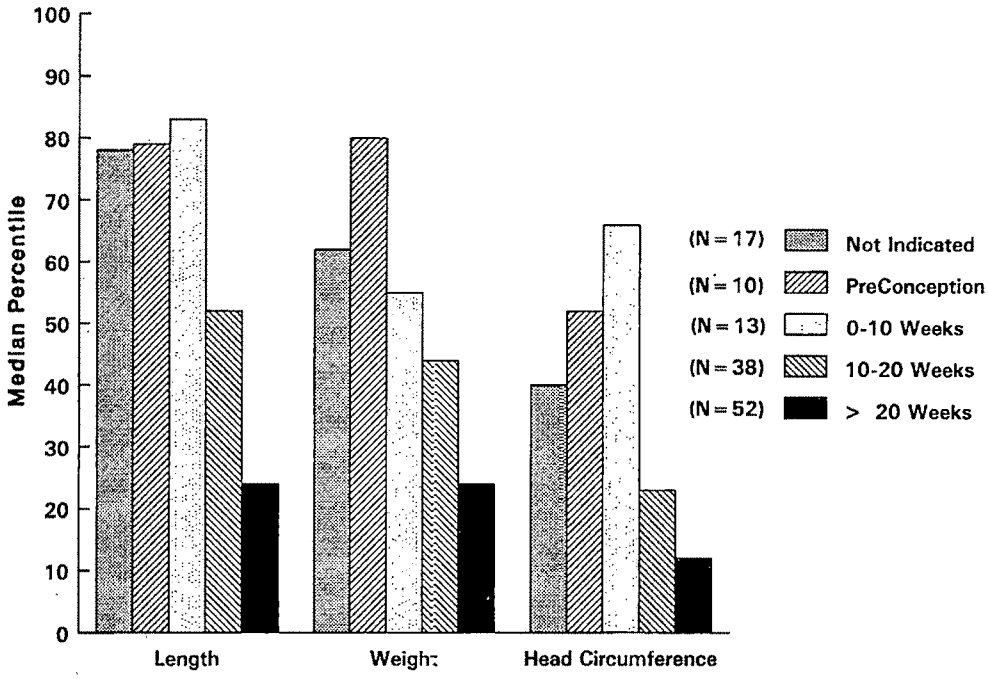


Fig. 3. Median birth measurement percentiles by gestational age at which maternal blood phenylalanine level <600 μmol/L (<10 mg/dl) was achieved.

Table VI. Delivery summary of hyperphenylalaninemic and control pregnancies

	Hyperphenylalaninemia		*Control*		
	n	%	*n*	%	*p Value*
Delivery method					
Vaginal	98	77	33	72	0.65
Cesarean section	30	23	13	28	
Multiple births	2	<2	2	4	0.65
Sex					
M	61	45	28	54	0.37
F	74	55	24	46	
Apgar test score, 1 minute					
2 to 5	8	6	4	9	0.83
6 to 8	71	56	26	57	0.92
9 to 10	48	38	16	35	0.85
Apgar test score, 5 minutes					
2 to 5	0	0	1	2	0.59
6 to 8	13	10	5	11	0.87
9 to 10	114	90	40	87	0.81
Birth measures <10th percentile					
Length	14	11	0	0	0.01
Weight	14	11	0	0	0.02
Head circumference	30	24	0	0	0.0001

women whose phenylalanine levels were >500 μmol/L (>10 mg/dl). The cardiac defects observed included ventricular septal defect, hypoplastic left ventricle, coarctation of the aorta, and tetralogy of Fallot. None of the women with hyperphenylalaninemia whose levels were ≤600 μmol/L (≤10 mg/dl) had infants with cardiac defects.

For this report, facial dysmorphism is defined as infants who had one or more abnormal facial features in an infant. Abnormal facial appearance included features such as epicanthal folds, prominent ears, long philtrum, upturned nose, micrognathia, and wide, depressed nasal bridge. The majority of infants born to affected mothers with hyperphenylalaninemia at all phenylalanine blood levels were noted to have at least one dysmorphic feature. The highest incidence (97%, 29 infants) was noted in women with phenylalanine levels ≥1200 μmol/L (≥20 mg/dl). The frequency of

Table VII. Birth measures of hyperphenylalaninemic offspring in relation to maternal smoking

	Positive smoking history				
	No		*Yes*		
	n	%	*n*	%	*p Value**
Birth length (percentile)					
<10th	7	7	5	15	0.24
10th to 50th	27	32	14	41	
>50th	50	60	15	44	
Birth weight (percentile)					
<10th	10	12	3	9	0.47
10th to 50th	31	36	19	56	
>50th	45	52	12	35	
Head circumference (percentile)					
<10th	14	17	12	35	0.046
10th to 50th	46	54	15	44	
>50th	25	29	7	21	

*Comparisons between birth measures <10th percentile and birth measures ≥10th percentile.

Table VIII. Congenital malformations observed in offspring of women with hyperphenylalaninemia

	Phenylalanine level					
	≥1200 μmol/L (≥20 mg/dl) (n = 30)		*600 to <1200 μmol/L (10.1 to 19.9 mg/dl) (n = 39)*		*<600 μmol/L (≤10 mg/dl) (n = 32)*	
	No.	%	*No.*	%	*No.*	%
Microcephaly	27	90	19	49	0	0
Intrauterine growth retardation	27	90	4	10	0	0
Facial dysmorphism	29	97	36	92	28	88
Cardiac defects	1	3	5	13	0	0
Ventricular septal defect	0	0	1	3	0	0
Coarctation of aorta	0	0	2	5	0	0
Coarctation, hypoplastic left ventricle	0	0	1	3	0	0
Tetralogy of Fallot	1	3	1	3	0	0
Inguinal hernia	0	0	1	3	1	3
Undescended testes	1	3	1	3	1	3
Hypospadias	1	3	1	3	1	3
Club feet	1	3	1	3	0	0
Syndactyly of finger	0	0	1	3	0	0
Kidney (duplicated collection system)	0	0	0	0	1	3

this disorder decreased in relation to a decrease in blood levels. Ninety-two percent (36 infants) and 88% (28 infants) of infants with mothers having intermediate and low blood levels, respectively, were found to have dysmorphic facies.

A number of minor anomalies were noted in the entire affected population regardless of the maternal blood phenylalanine concentration. These included 2 infants with inguinal hernias, 3 with undescended testes, 3 with hypospadias, 2 with club feet, 1 with syndactyly, and 1 with a renal anomaly: a duplicated collection system.

Comment

The success of dietary therapy in arresting the neurologic deficits caused by hyperphenylalaninemia has inadvertently produced an increasing number of non-hyperphenylalaninemia mentally retarded offspring. Currently there are >1600 known and tracked women with hyperphenylalaninemia in the United States and Canada who are of childbearing age. Taking into account the numbers of unknown women with hyperphenylalaninemia, including those born before newborn screening for phenylketonuria or who immigrated to the United States from countries with no newborn screening, conservative estimates have suggested that there may be 5500 fecund women with hyperphenylalaninemia in the United States who are at risk of giving birth to an affected offspring.[6] The possibility has been raised that after only one generation, given average reproductive rates, the incidence of phenylketonuria-related mental retardation could return to the level existent before national screening and treatment.[7] The threat of this problem has thus demanded

a large, well-designed, well-controlled investigation of the problem and its potential prevention, such as that developed by the National Maternal Phenylketonuria Collaborative Study. This study is especially unique in that, to ensure its success, it has required the active participation a large number of individual institutions and the cooperative efforts of a multidisciplinary medical team that includes obstetricians. With the advancement of dietary therapy phenylketonuria has routinely been categorized as a medical disorder of children, the treatment of which has interwoven the efforts of pediatricians, nutritionists, psychologists, and public health personnel. Obstetric contributions regarding this disorder have been notably limited. However, with the recognition of the teratogenic effects on the fetus and related interventions, the need for increased involvement by the obstetrics community in maternal phenylketonuria is clearly evident. One of the goals of this report is to alert the maternal-fetal medical community of the identification, antenatal and intrapartum effects, and treatment of this disorder.

Approximately two thirds of the anticipated enrollment of subjects with hyperphenylalaninemia into the Collaborative Study has been achieved. It is anticipated that the enlistment phase of the study will continue until the end of 1994 to achieve the desired sample of 200 mother-infant dyads. Although definitive conclusions concerning this investigation are forthcoming, early analyses of the available data as presented in this preliminary report suggest several findings and considerations.

As indicated by the results of this study, dietary control is essential for optimal pregnancy outcome. An important consideration in dietary management is the provision of proper nutrition to ensure an adequate maternal weight gain for normal fetal growth and development. The mean weight gain of the women with hyperphenylalaninemia demonstrated that affected women achieved their recommended weight during pregnancy and maintained a strict phenylalanine-restricted diet. However, the lowest weight gain was seen in women who were preconceptually <90% of the recommended weight for their height. Further analysis of the data is needed to determine whether the incidence of intrauterine growth retardation is related to inadequate weight gain in this specific weight class.

Observations of the occurrence of congenital malformations have indicated that a minimal teratogenic effect of hyperphenylalaninemia occurred when maternal blood phenylalanine levels were maintained at <600 μmol/L (10 mg/dl) for all three trimesters of pregnancy. Comparison of the current data with an international review of women with untreated hyperphenylalaninemia[5] confirms the importance of maintaining minimal blood phenylalanine levels. The incidence of microcephaly was found not to be significantly different between the treated and the historic untreated populations for phenylalanine levels of ≥1200 μmol/L (≥20 mg/dl) and levels between 600 and <1200 μmol/L (10.1 to 19.9 mg/dl). The treated hyperphenylalaninemia pregnancies of the current study with blood levels <600 μmol/L (<10 mg/dl) did have a significantly ($p = 0.007$) lower incidence of microcephaly than the untreated patients in the historic study. In terms of the occurrence of congenital heart defects, however, no significant differences were noted in any classification of phenylalanine blood levels when comparisons were made between the two studies. Because the desired population size has not yet been achieved, it is premature to say whether this is a valid observation or a statistical β error.

Initiating diet therapy during the third trimester of pregnancy appears to have minimal effect on fetal outcome. As indicated in the observations made on birth measurements, optimal treatment levels (<600 μmol/L (<10 mg/dl)) should be achieved by 8 to 10 weeks' gestation, with trimester levels averaging ≤360 μmol/L (≤6 mg/dl). Dose-related effects of phenylalanine could be observed in the higher incidence of birth length, weight, and particularly head circumference at <10th percentile in women with hyperphenylalaninemia who were not well controlled during pregnancy.

A further finding that appears to have a variation of effect dependent on phenylalanine level is facial dysmorphism. Study findings continue to demonstrate an increasing incidence of dysmorphic features with increasing maternal phenylalanine blood levels as initially reported by Rouse et al.[8] A similar incidence of dysmorphic facies has been reported by Lipson et al.,[9] who attribute the facial appearance of offspring with hyperphenylalaninemia to facial bone hypoplasia.

The women with hyperphenylalaninemia had a higher incidence of fetal loss than the control group. Although the frequency of spontaneous abortion (17%) in the hyperphenylalaninemia group appears to be excessive in comparison with no loss in the control women, the lower incidence of spontaneous abortion in the control group could be attributed to sampling differences. Only 4% of the control population, as compared with 31% of the hyperphenylalaninemia group, were enrolled in the study by 4 weeks' gestation, a period normally associated with a significant rate of early, often undetected, abortion.[10] Even without this consideration, the spontaneous abortion rate of 17% in the hyperphenylalaninemia group may well be within the limits of the average spontaneous loss rate of 40% in some populations.[10-12] Further, there was no appreciable difference noted in a comparison of the incidence of elective termination of the study population (19%) and the general population (33%).[13]

The relatively young age of the women with hyperphenylalaninemia at enrollment in the study is not an unexpected finding. National newborn screening for phenylketonuria was instituted in Canada and most of

the United States by 1965. The first generation of identified and treated infants with phenylketonuria, now between the ages of 15 and 25 years, was targeted for this study. Obstetric management of the hyperphenylalaninemia-affected group between the ages of 15 and 19 years, representing 27% of the sample, presented a number of challenges. Pregnancies in very young women have been reported to have an increased incidence of antenatal and intrapartum complications. However, the preliminary results of the study do not demonstrate an increase of high risk obstetric conditions in the hyperphenylalaninemia group when compared with the older control group.

A further consideration in this younger sample is the incidence of sexual activity. A number of surveys of women between the ages of 15 and 19 years have shown this age group to be sexually active with limited use of birth control.[14, 15] It has been reported that 42% of all never-married 15- to 19-year-old adolescent girls have experienced sexual intercourse, with two thirds either inconsistently or never using some form of contraception.[15] A recent report by Waisbren et al.[16] concluded that sexual activity among adolescents and young women with phenylketonuria was comparable with that of their acquaintances who did not have phenylketonuria and with young women with diabetes, thus emphasizing the risks for pregnancy in this population. These statistics, in conjunction with the hazards of maternal phenylketonuria syndrome, indicate the importance of education in the hyperphenylalaninemia population. Known patients with hyperphenylalaninemia should be counseled about dietary control, the possible consequences of pregnancy, and available methods of birth control. Nevertheless, they are not more fastidious in the use of conventional birth control than their peers.[16] Consequently it seems reasonable to consider the use of the new, long-lasting subdermal implant, Norplant (levonorgestrel). Norplant has been shown to be effective for up to 5 years after implantation, with a reported pregnancy rate of <1%.[17] The effectiveness and convenience of Norplant make it an ideal method of birth control for the younger group of women with hyperphenylalaninemia. It may also be an ideal contraceptive device for older women with hyperphenylalaninemia of childbearing age who were born before newborn screening and treatment and may be intellectually impaired.

Preconceptual education of women with hyperphenylalaninemia should also include information concerning the risks of tobacco use during pregnancy. Although studies have previously documented an increased incidence of low-birth-weight infants in women without hyperphenylalaninemia who smoked during pregnancy, maternal smoking appears to be especially hazardous in hyperphenylalaninemia women. The hyperphenylalaninemia study population who had infants with head circumferences <10th percentile had a significantly increased history of smoking. Thus it appears that maternal smoking provides a compounding effect in a population already at risk for infants with a decreased head size. Further investigation of this issue will be required.

The preliminary obstetric findings of the study suggest that the teratogenic effects of hyperphenylalaninemia can be avoided with preconceptual or early gestational dietary control maintained throughout pregnancy. Although dietary therapy is essential in the management of maternal phenylketonuria, it is of little benefit to undiagnosed women with hyperphenylalaninemia. Newborn screening has determined the incidence of phenylketonuria is about 1 in 15,000 and has led to the identification of phenylketonuria in most women under the age of 25 years. However, Hanley et al.[18] have estimated that the incidence of undiagnosed maternal phenylketonuria is about 1 in 30,000. The majority of women with undiagnosed phenylketonuria were born before the initiation of newborn screening. Also included in these numbers are women previously diagnosed with phenylketonuria who have been lost to medical follow-up and women with phenylketonuria who have emigrated from countries without a newborn-screening program.[18] Elimination of the devastating congenital effects associated with maternal phenylketonuria is not possible until all women with phenylketonuria are identified and treated. Several strategies to accomplish this task have been recommended. Among these are the establishment of a national phenylketonuria registry in association with newborn-screening laboratories to keep updated records on patients with phenylketonuria.[19-21] Various phenylketonuria rescreening programs have also been suggested. At a minimum, screening should be performed with any woman who has had a mentally retarded or microcephalic child.[18, 22] Further, screening programs could be initiated at family planning clinics and prenatal clinics[23] with a cost-effectiveness that could approach that in routine newborn screening.[21]

The results of this study are preliminary and further study is ongoing. These data demonstrate the importance of preconceptual counseling and early entrance into a prenatal care program to reduce the incidence of congenital malformations and developmental disabilities in the offspring of women affected by hyperphenylalaninemia.

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Editor's note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Joe Leigh Simpson, Memphis, Tennessee (Fellow). A vexing problem in human genetics and obstetrics is that of pregnancy in women with hyperphenylalaninemia or phenylketonuria who were treated in childhood but who stopped their diets when they became adolescents or adults. For over a decade it has been recognized that offspring of such individuals are at increased risk for abnormal development. To address this, a collaborative effort involving most phenylketonuria centers in the United States has been underway since 1984. Dr. Platt has been pivotal to this effort, as is evident by his peers selecting him to present this preliminary report on the obstetric outcome of subjects recruited during the first 6 years of the study.

The project is certainly a daunting one. Phenylketonuria is rare; therefore, the task of identifying a sufficient sample of individuals with phenylketonuria who become pregnant is formidable. Yet we have heard excellent data. Optimal fetal outcome was observed where maternal phenylalanine levels were <10 mg/dl by 8 to 10 weeks' gestation. In microcephaly a dose response curve was observed; with respect to phenylalanine levels, all six cardiac anomalies occurred in women whose levels were >10 mg/dl.

I have a few comments about which Dr. Platt's thoughts would be appreciated:

Comparability. Given the enormous amount of effort expended in recruiting the subjects with phenylketonuria, it is disappointing that comparable effort did not go into the controls. Only 70 controls have been identified: 52 office controls, 8 familial controls, and 10 heterozygous mothers. In aggregate, these women produced 51 completed pregnancies. Moreover, there were substantial differences between the controls and subjects with respect to maternal age and exposure to alcohol and cigarettes.

An indication of the problem with controls is that "no fetal wastage was observed in the control group of the study." Naturally the difference between the phenylketonuria and control groups was statistically significant ($p = 0.0002$). Yet the authors themselves note in their Comment that the comparison is invalid. Weeks of observations are different in the phenylketonuria group and controls, fewer in the latter. The proper way to analyze fetal wastage would involve a life-table approach that would take into account the differing numbers of weeks of observations in the two groups.

Given that this study is ongoing, I hope that greater effort can go into recruiting controls. Perhaps sisters-in-law could be more aggressively sought; of course, recruiting unaffected sisters would have the advantage of observing outcome in more heterozygotes.

Assessment of anomalies. I was puzzled by the higher cardiac anomaly rate (5 of 39, 13%) in women whose phenylalanine levels were 10.1 to 19.9 mg/dl than in those with levels >20 mg/dl (1 of 30 or 3%).

Perhaps this anomaly reflects the trimester in which phenylalanine was measured, which would bear on organ systems that complete development by 12 weeks. Specifically, congenital heart disease should be correlated only with first trimester values. Were they?

Another difficulty relates to assessing facial dysmorphism and minor anomalies. In my opinion there are nearly insurmountable problems in assessing these in collaborative studies because there are few consensus definitions for such semiquantitative anomalies. Some

assurance concerning unbiased assessment of facial dysmorphism would also be nice. That is, was the examination done by an observer unaware of the phenylalanine level? If not, the observer might search more vigorously for anomalies in hyperphenylalaninemia subjects.

Health care delivery problems. I would appreciate the authors' opinion about the responsibility of the patients with phenylketonuria for their own care. We worry that patients with phenylketonuria are not being followed up, and contemplate organizing rescreening programs for that purpose. Yet surely the patients (who had to be intelligent enough to sign a probably complicated informed consent) were aware that they had phenylketonuria as children. (Incidentally, the selection bias of excluding patients with lower intelligence quotients is a general concern.) Surely women with phenylketonuria could not have forgotten the terrible phenylalanine-free diet. Why, then, was not a primary care physician aware of the history before pregnancy? I understand the concept of denial but submit there *is* a patient responsibility. Without such I would be very pessimistic about decreasing anomalies in offspring of mothers with phenylketonuria. After all, genetic screening programs in the nonpregnant adult population have not universally met with rousing success. What is the authors' opinion about the impact and likely success of a system of continued tracing, to say nothing of success of reimplementing diets? A related question is whether they would treat mild phenylketonuria patients who never required treatment during childhood.

In conclusion, I feel privileged to have an opportunity to discuss this long-awaited study by a physician who has contributed greatly not just to ultrasonography but to other important obstetric areas. My disappointment with the control group and other minor concerns do not diminish my general enthusiasm for the major effort presented here.

Dr. Allan P. Killam, Durham, North Carolina. There has been a controversy about NutraSweet sweetener that breaks down into phenylalanine and methyl alcohol. An investigator said in the press that to switch to diet drinks as opposed to regular drinks might expose women with phenylketonuria or even carriers of phenylketonuria to greater incidence of mental retardation in their children.

The makers of NutraSweet sweetener have countered that the amount of phenylalanine in a diet drink is minuscule compared with a banana, a glass of milk, or a hamburger and that this is not a cause of a concern for people with misdiagnosed or undiagnosed phenylketonuria or carriers.

I just wanted to know what is the order of magnitude of phenylalanine in diet drinks, and does this worry that the Centers for Disease Control has promulgated have any basis in science.

Dr. Rudy E. Sabbagha, Chicago, Illinois. How severe was the microcephaly? Usually we make a distinction as to whether head size is 2 SDs below the mean and really not associated with severe future adverse outcome or whether head size is in the range of 3 to 5 SDs below the mean.

Sometimes in ultrasonography we have difficulty diagnosing microcephaly at 28 weeks' gestation. Were these diagnoses made after or before 28 weeks, and how many of the cardiac defects were diagnosed antenatally?

Dr. Robert C. Cefalo, Chapel Hill, North Carolina. How did you control for supplementation for the patients with hyperphenylalaninemia?

Dr. Platt (Closing). Dr. Simpson's question deals with the comparability of the patients with phenylketonuria with the controls and the problems that we had. I believe that during the course of my presentation I, too, illustrated the problems we had with the controls. Since our last review of the entire study we have undertaken a greater emphasis to enroll appropriate controls so that we would have a comparable group at the end of the recruitment of 200 patients with phenylketonuria. This includes using sister cohorts as you have suggested, which we also think is a very good idea.

In terms of our anomaly assessment and interpretation, I think your observations regarding congenital heart disease are also correct. It does appear to be a trimester-related phenomenon in which ongoing changes occur that may not be reflected or seen until later. Clearly, if there is going to be an impact in the reduction of problems such as congenital heart disease, it has to occur early in the first trimester. The number of cases thus far with congenital heart disease is too small to tell whether there is a dose relationship week by week of pregnancy. I hope that by the time we reach our desired sample size of 200 we will have better information with regard to levels and early pregnancy.

In terms of fetal dysmorphism, first let me tell you that all measurements were made without anybody being aware of either the mean phenylalanine levels or the highest peak levels. All data were recorded on a particular code sheet in the cooperating centers and sent to one of the investigators, Dr. Bobbye Rouse, for the formal analysis of the presence of fetal dysmorphism. I think it is quite clear that the effect of some of these changes are clearly related to phenylketonuria. This is not different for other syndromes such as fetal alcohol. I guess it is in the eyes of the beholder.

In terms of the impact on health care and the problems seen with phenylketonuria, I believe there are some sixteen hundred known women with phenylketonuria today whom we are aware of through the various pediatric metabolic centers that monitor these women. There may be >5000 women who may also have hyperphenylalaninemia who may not have been screened earlier or who now have higher levels. The impact here is on education. Some of the patients who get pregnant are seen by primary care providers who are certainly not as aware of the hazards of phenylketonuria because they do not see this disorder in their practices very often. None of these women were seen in study centers; some were seen 200 miles away from the centers to which they had been referred for certain

parts of their treatment. The primary care providers in some instances had very, very little knowledge of phenylketonuria and were unaware of it until the patient, sometime later in the pregnancy, informed them of it. The care provider then got in contact with the centers. With increasing education and by exposure to our obstetric literature one of the problems of the collaborating centers may be alleviated.

As to Dr. Killam's question about NutraSweet sweetener, I think normal patients are at no great risk. On the other hand, in undiagnosed hyperphenylalaninemic women there certainly is a risk. This argument would be the basis for a study to look at a selected population group, perhaps women who have recently moved to the United States in areas such as Los Angeles who have not previously been screened. These women would be studied to determine the impact of substances such as NutraSweet sweetener on their phenylalanine levels.

With regard to Dr. Sabbagha's questions about microcephaly and the timing of diagnosis, some of the cases were clearly diagnosed in utero. We have previously recognized the difficulty in diagnosing microcephaly early in pregnancy. In the newborn 2 SDs below the mean were used as the definition for this study. I would agree with you that we have to wait for more obvious signs, even 3 SDs in the in utero period, because they do not often show themselves till after 28 weeks' gestation. In terms of the cardiac malformations, half of the defects were picked up. The other half were missed. I do not think that that is surprising. It is probably a very good yield considering where all these patients have come from.

Dr. Cefalo's question was how we controlled for vitamin nutrients. Every patient entered in the study underwent counseling with nutritionists who were very much involved in this particular program.

Postmenopausal steroid replacement with micronized dehydroepiandrosterone: Preliminary oral bioavailability and dose proportionality studies

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Memphis, Tennessee, and Santa Monica and Torrance, California

OBJECTIVES: Because dehydroepiandrosterone may protect against neoplasia, osteoporosis, and cardiac disease, we investigated the bioavailability of oral micronized dehydroepiandrosterone, anticipating its adjunctive use in postmenopausal steroid replacement.

STUDY DESIGN: Eight postmenopausal women randomly received either a placebo or 150 or 300 mg of oral micronized dehydroepiandrosterone in a lipid matrix. Serum dehydroepiandrosterone, dehydroepiandrosterone sulfate, testosterone, and estradiol were measured periodically over the 12 hours after each dose. All treatments, all doses, and mean serum dehydroepiandrosterone, dehydroepiandrosterone sulfate, and testosterone were compared with analysis of variance for repeated measures and Newman-Keuls a posteriori test of statistical significance.

RESULTS: Mean peak steroid concentrations after 150 mg (300 mg) doses were dehydroepiandrosterone 1617 (2639) ng/dl, 7 (11.5)-fold above placebo; dehydroepiandrosterone S 1185 (1688) μg/dl, 14 (20)-fold above placebo; and testosterone 183 (311) ng/dl, 4 (7)-fold above placebo. Estradiol concentrations remained <20 pg/ml, but androgen concentrations rose by 1 hour and remained elevated through the twelfth hour. Peak androgen concentrations and areas under the curves exhibited proportionality with both doses. A testosterone radioimmunoassay with celite chromatography revealed a 300% overestimation for testosterone in the direct-assay method used in this study. Thus after appropriate readjustment maximum testosterone concentrations were observed consistently within physiologic premenopausal ranges after the 150 mg dose.

CONCLUSIONS: Micronized dehydroepiandrosterone may provide a steroidal postmenopausal replacement that is adjunctive to estrogens and worthy of further investigation. (AM J OBSTET GYNECOL 1992;166:1163-70.)

Key words: Dehydroepiandrosterone, menopause, replacement, micronized dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) and its sulfated conjugate dehydroepiandrosterone sulfate (DHEAS) are weak androgens secreted by the adrenal cortex in amounts exceeding cortisol.[1] In both men and women production of DHEA and DHEAS rises sharply before puberty and peaks between 20 and 30 years of age independent of cortisol.[2] DHEA DHEAS production and adrenal corticotropin responsiveness decline with advancing age.[3] Ovarian failure at any age accelerates this decline.[4, 5]

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Because DHEA and DHEAS were previously considered to be relatively inert circulating adrenal steroidogenic intermediates, the implications of a putative adrenopause were ignored. An increasing body of literature, however, in both human subjects and experimental animals, indicates that physiologic DHEA may protect against neoplasia,[6-8] enhance insulin action,[9, 10] protect against osteoporosis,[11-13] increase immune system competence,[14, 15] and offer (at least in men) cardiac protection.[16] Furthermore, pharmacologic DHEA may exert salutary effects on serum lipoprotein profiles, muscle mass, and body fat percentage in young men.[17] Given the impact of these observations on the quality of postmenopausal life, investigation of DHEA as a possible steroid replacement is timely.

Endogenous DHEA and DHEAS production and conversion contribute modestly to the pool of circulating biologically active androgens and estrogens in women after menopause.[18] With pharmacologic DHEA, however, immediate substantial increases in circulating testosterone and dihydrotestosterone are ob-

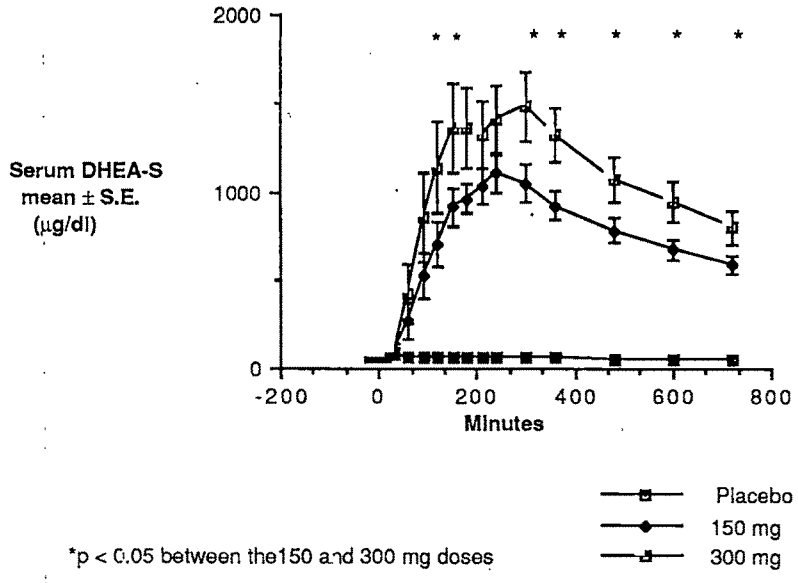


Fig. 1. Mean serum DHEAS concentrations after oral administration of placebo or 150 or 300 mg of micronized DHEA (n = 8).

Table I. Pertinent screening data on subjects (n = 8) enrolled in protocol

Parameter	*Mean*	*Range*
Age (yr)	60	48 to 69
Weight (lb)	138	131 to 151
Percentage of ideal body weight	105	93 to 115
Gravidity	—	2 to 5
Parity	—	2 to 7
Testosterone (ng/dl)	45	9 to 60
DHEAS (μg/dl)	55	9 to 11
Morning cortisol (μg/dl)	14	8.4 to 22

served, with estrone and estradiol increasing modestly after 2 to 4 weeks of chronic administration.[19] If there were a reliable method of delivering DHEA at replacement levels, postmenopausal administration of this hormone would be an attractive adjunct to estrogen replacement. Our study investigates replacement doses of oral micronized DHEA delivered in a lipid matrix as a potential strategy directed toward this goal.

Material and methods

With institutional review board approval, eight postmenopausal women were recruited for this study. Medical histories and physical examinations, complete blood cell count, liver, renal, and thyroid function tests, and fasting serum insulin, cortisol, testosterone, and DHEAS concentrations were completed for all eight women. Liver function tests were repeated on completion of the study. Table I lists screening data for the eight participants. Inclusion criteria included body weight between 80% and 120% of the ideal defined by the Metropolitan Life tabular method and at least 6 months of amenorrhea with menopausal symptoms. Any subjects with <1 year of amenorrhea had menopausal status reconfirmed by follice-stimulating hormone >40 mIU/ml. Subjects were either receiving no estrogen replacement therapy (n = 6) or had stopped taking replacement estrogens for at least 2 weeks before and for the duration of the study (n = 2). None smoked cigarettes or took other medication.

The eight subjects were randomly allocated to receive 150 or 300 mg of micronized DHEA or a placebo tablet of identical formulation, each in succession separated by ≥1 week between treatments. Pharmacopeia-grade DHEA (Sigma Chemical, St. Louis) was micronized to <10 μm particles in 90% of the particles examined. The micronized DHEA was mixed with a wax vegetable oil matrix and compressed with a silica-based excipient into a tablet containing 150 mg DHEA (Belmar Pharmacy, Lakewood, Colo.). The patients were fasting when they were admitted to the University of Tennessee, Memphis, Clinical Research Center at 10:00 AM; at that time a heparin lock flush solution was intravenously established in an antecubital vein. DHEA or placebo doses were administered at time 0, and blood samples were drawn at −20, −5, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 480, and 720 minutes. Each patient had a light standard meal after 120 minutes if desired. Serum was separated and stored for assay for DHEA, DHEAS, testosterone, and estradiol.

Testosterone and DHEAS concentrations were measured with commercial kits that assay directly with iodine 125–label (Pantex Ltd., Santa Monica, Calif.). DHEA was measured by a commercial tritiated extrac-

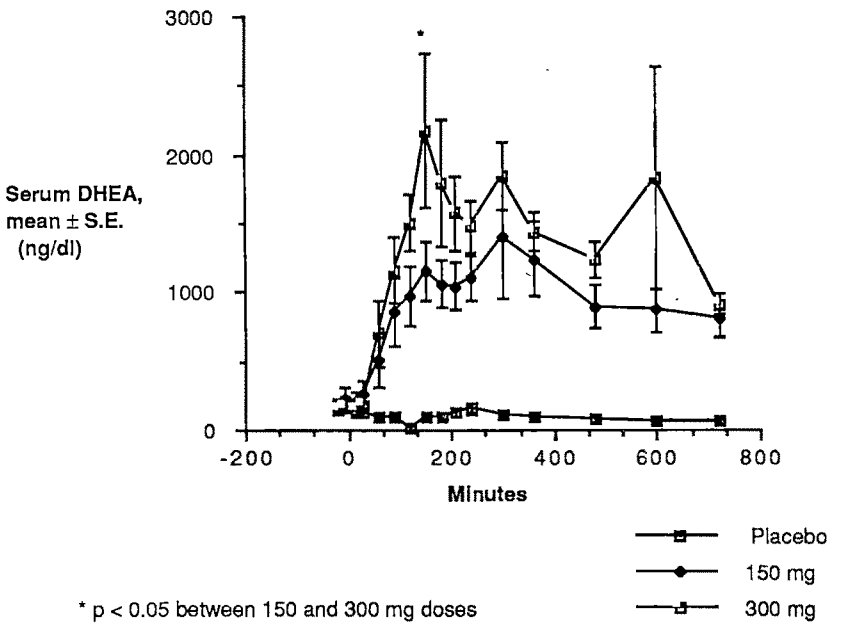


Fig. 2. Mean serum DHEA concentrations after oral administration of placebo or 150 or 300 mg, of micronized DHEA (n = 8).

tion assay (Wein Scientific, Succasunna, N.J.). The interassay variation of the DHEAS, DHEA, and testosterone assays was 8%, 6.5%, 9%, and 8.5%, respectively. The specificity of the testosterone assay was further evaluated by taking a selection of 24 samples from this study with a wide range of testosterone values and reassaying after using a tritiated method, including a preparatory celite chromatography step as described previously.[20] Estradiol was measured by a tritiated radioimmunoaassay with preparatory celite chromatography.[20, 21]

At all time intervals, treatments, and doses mean serum DHEA, DHEAS, and testosterone were compared with analysis of variance for repeated measures and Newman-Keuls a posteriori tests of statistical significance. Peak serum concentrations of all three steroids obtained with each treatment arm were identified, and the mean values were determined. The mean peak values were compared for each steroid and for each treatment arm by the same tests of statistical significance. For each treatment arm for each subject, the area under the serum concentration-time curve for DHEA, DHEAS, and testosterone was calculated by the method of summative trapezoids. Areas under the serum concentration-time curve were derived from all time-concentration curves for the eight patients, and the mean areas under the serum concentration-time curve were calculated. These were then compared statistically within each of the three steroid measurements and between the doses given. The DHEA dose proportionality was determined by comparing areas under the serum concentration-time curve for each steroid and mean peak levels against dose. If the resulting plot was relatively linear, dose proportionality was said to exist. All statistics were done using a statistical software package (Statistical Analysis Systems, Inc., Cary, N.C.).

Results

Mean concentrations of DHEA, DHEAS, and testosterone over the 12 hours after administration of the two micronized DHEA doses or the placebo tablet are presented in Figs. 1 through 3. For all steroids significantly increased levels are seen 1 hour after administration of the DHEA except for estradiol. The estradiol level was <20 pg/ml in 24 samples tested, corresponding to both baseline and highest androgen levels. Furthermore, estradiol showed no systematic increase in concentration within these low ranges. Peak androgen levels were seen 3 to 5 hours after administration.

Mean peak concentration for each steroid for each subject and each treatment is listed in Table II. Similarly, Table III lists the mean of the areas under the serum concentration-time curve for each patient for each steroid as achieved with each of the three treatments. Mean peak concentrations for each of these steroids at 150 mg (300 mg) doses were DHEA 1617 (2639) ng/dl, 7 (11.5)-fold above placebo; DHEAS 1185 (1688) μg/dl, 14 (20)-fold above placebo; and testosterone 183 (311) ng/dl, 4 (7)-fold above placebo. Significant differences existed between peak concentrations of DHEAS, DHEA, and testosterone attained with placebo versus both DHEA doses. Significant differences also existed between the DHEAS and testosterone peaks attained with the two-dose formulations. A sim-

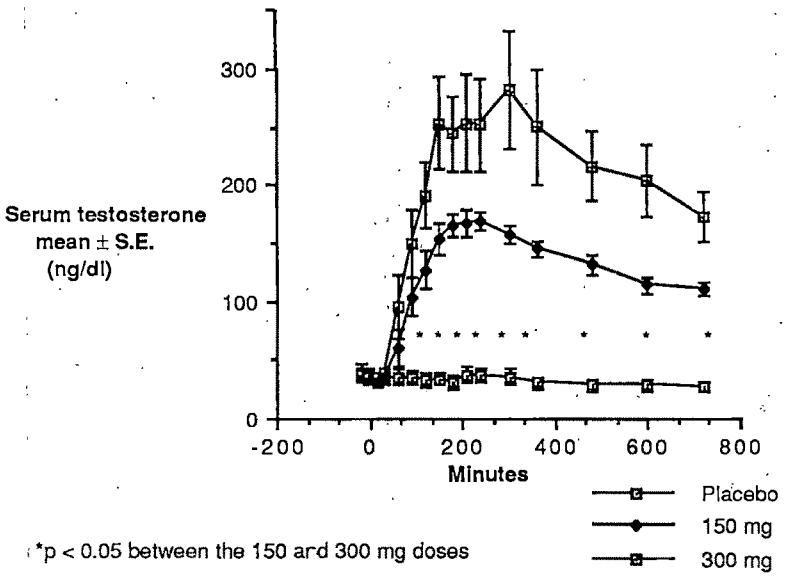


Fig. 3. Mean serum testosterone concentrations after oral administration of placebo or 150 or 300 mg of micronized DHEA ($n = 8$). These values were measured with a direct ^{125}I radioimmunoassay; they overestimate the actual testosterone by approximately 300%.

Table II. Time-standardized mean peak serum DHEAS, DHEA, and testosterone concentrations after oral administration of micronized DHEA

	Serum steroid concentration		
	DHEAS (μg/dl)	*DHEA (ng/dl)*	*Testosterone (ng/dl)*
Placebo	83 ± 713	228 ± 54	45 ± 7
DHEA			
150 mg	1185* ± 110	1617* ± 429	183* ± 10
300 mg	1688† ± 215	2639* ± 510	311† ± 52

Amounts are mean ± SEM.
*$p < 0.05$ from placebo peak.
†$p < 0.05$ between peaks at 150 and 300 mg.

ilar pattern of differences is seen when the mean areas under the serum concentration-time curve for each dose preparation are compared. Dose proportionality (not shown) analysis reveals a linear plot, indicating that for this particular preparation DHEA dose proportionality exists. Analysis of the samples taken 24 hours after 150-mg dose administration in two patients revealed that the DHEAS concentrations remained approximately three times placebo levels, and the testosterone values had nearly returned to preadministration levels.

Concentrations determined in this study by the direct ^{125}I radioimmunoassays for testosterone were compared with those obtained by the tritiated assays that use preparative celite chromatography and whose specificity for these steroids was documented previously.[20] Fig. 4 compares the two methods by linear regression and demonstrates that the direct assay systematically overestimates testosterone by approximately 300%.

Liver function tests obtained after completion of each arm were within normal limits in seven of the eight subjects. In one of the participants, however, there was a rise in liver enzymes at the end of the study, with clinical hepatitis developing 2 weeks after completion. This complication was associated with preexisting antinuclear antibody titers and was resolved completely.

Comment

This study demonstrates rapid absorption and biotransformation of oral micronized DHEA to DHEAS and to testosterone proportionate to the doses given. Thus single 150 and 300 mg doses produced, respectively, supraphysiologic maximum concentrations of DHEA (1617 to 2639 ng/dl, 7 to 11.5 to fold × placebo) and DHEAS (1185 to 1688 μg/dl, 14- to 20-fold × placebo) with comparatively modest elevation of testosterone (183 to 311 ng/dl, 4- to 7-fold × placebo). When testosterone is adjusted to one third after chro-

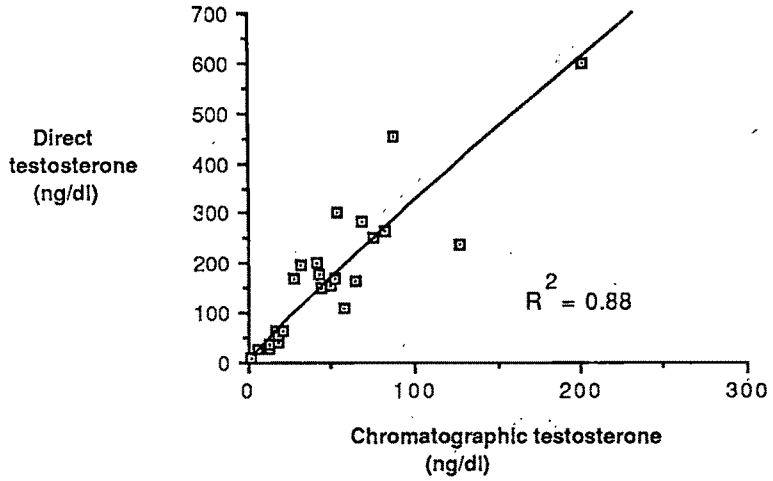


Fig. 4. Testosterone concentrations determined by direct ^{125}I radioimmunoassay versus tritiated assay with chromatography. The direct assay overestimates testosterone by 300%.

Table III. Mean areas under the serum concentration-time curve for DHEAS, testosterone, after oral administration of micronized DHEA ($n = 8$)

	Area under serum concentration-time curve		
	DHEAS (μg/min/dl)	*DHEA (ng/min/dl)*	*Testosterone (ng/min/dl)*
Placebo	42,415 ± 8,768	17,198 ± 14,063	22,753 ± 3,646
DHEA			
150 mg	540,027* ± 35,757	683,810* ± 125,124	91,193* ± 3,994
300 mg	757,493*† ± 89,509	903,735* ± 93,570	149,262*† ± 20,643

Amounts are mean ± SEM.
*$p < 0.05$ from placebo peak.
†$p < 0.05$ between peaks at 150 and 300 mg.

matographic assay, mean testosterone concentrations remain within normal premenopausal levels throughout the 12 hours after the 150 mg dose. By extrapolation from Figs. 1 through 3, 50 to 75 mg daily of this DHEA preparation may be expected to approximate the premenopausal adrenal androgen milieu. Thus the premenopausal patient would accrue benefits of premenopausal DHEA concentations without incurring deleterious effects from excess of potent androgen metabolites.

To our knowledge this is the first systematic investigation of DHEA given to postmenopausal women in replacement doses. However, DHEA administered in much higher doses (1600 mg per day) to obese postmenopausal women was associated with deleterious androgenic effects. In that study, six obese postmenopausal women treated for 28 days developed insulin resistance and decreased sex hormone–binding globulin in association with substantial increases in testosterone and dihydrotestosterone.[19] We postulated that lower doses with a strategy to bypass or overwhelm hepatic metabolism could potentially deliver a more physiologic androgen environment.

Normally, adrenal androgens are secreted into the systemic circulation. Therefore oral administration of DHEA with its consequent hepatic first-pass effect is unphysiologic. Our strategy combines micronized DHEA particles for rapid portal entry to overwhelm hepatic metabolism of the native steroid with a lipophilic vehicle designed to bypass the liver via lymphatics. Previous studies of oral DHEA administration used formulations of plain, milled, crystalline DHEA with no matrix.[17, 19, 22, 23] This form of DHEA, theoretically subject to substantial hepatic first-pass effect, produces high conversion of DHEA to biologically active androgens and concomitantly reduces the native DHEA bioavailability.[19] Our approach is based on a report by Kincl et al.[24] in which the bioavailability of native progesterone was significantly increased by administration with a cholesterol fatty acid ester.[24] Further studies in rats by the same authors revealed enhanced lymphatic absorption and transport with the fatty acid preparation, thus bypassing liver metabolism.[24, 25] Expanding on this principle we combined DHEA, micronized to <10 μm particles, with a lipid matrix. With micronization we sought to overcome some first-pass effect by

rapid portal entry of steroid. With the lipid matrix we hoped to bypass the liver via the lymphatic system.[54] By rough comparison administration of DHEA in this study produced higher DHEA and comparable DHEAS and testosterone concentrations, with one third of the dose of milled, crystalline DHEA reported elsewhere.[19] We made no direct comparisons in this preliminary study, which was designed only to examine dose effects.

In this single-dose study serum estradiol did not increase. A previous multidose study revealed both estrone and estradiol increase after 3 weeks of therapy to 40 to 50 pg/ml and 30 to 40 pg/ml, respectively.[19] Conversion of DHEA to androgens and further aromatization to estrogen may be induced with chronic DHEA therapy not evident in this single-dose study. Thus postmenopausal women given DHEA may not only benefit by the specific salutary effects of this hormone; they also may require less or no estrogen.

This preliminary study explores the possibility of a physiologic postmenopausal adrenal androgen replacement regimen. Given the numerous reported benefits of DHEA in animal and human studies, postmenopausal replacement is intuitively attractive and worthy of further investigation.

We thank Charles Hakala, RPh, of the Belmar Pharmacy, Lakewood, Colorado, for preparing the micronized DHEA tablets.

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Editor's note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Robert B. Jaffe, San Francisco, California (Fellow). The reasons for the existence of such high concentrations of DHEA during fetal, peripubertal, and adult life have remained enigmatic and intriguing for

many years. So, too, has the decline of DHEA and DHEAS with aging.

During intrauterine fetal life, DHEAS is quantitatively the major androgen secreted by the fetal adrenal gland. It is produced principally by the unique fetal zone of the gland[1] and, after 16-hydroxylation in the fetal liver and adrenal gland, is used after further metabolism as a substrate for placental estrogen. The production of DHEAS by the fetal adrenal gland is stimulated by corticotropin.[2] A putative adrenal androgen stimulating hormone distinct from corticotropin did not stimulate fetal DHEAS production.[3] Whether DHEA and DHEAS have other functions during intrauterine life awaits further elucidation.

After birth DHEAS increases significantly before puberty, an event known as adrenarche. This is associated with the initiation of sex hormone–dependent hair growth and is likely independent of menarche. Concentrations of DHEAS rise to peak levels during the third decade and then gradually decline. Some have referred to the diminution of DHEAS production as the adrenopause.

As indicated by the authors, several beneficial effects of DHEA administration have been suggested in the last several years. These have included inhibition of neoplasia, decrease in cardiovascular disease, and prevention of osteoporosis. However, administration of DHEA in different vehicles and for more prolonged periods has produced changes associated with the polycystic ovarian syndrome, namely increased insulin resistance, decreased sex hormone–binding globulin, and significant increases in testosterone and its 5α-reduced reduced metabolite, dihydrotestosterone.[4] Thus long-term administration of DHEA should be approached with caution.

A literature search on DHEA reveals that in addition to those effects, DHEA also has been reported to stimulate gene expression in the prostate gland,[5] stimulate growth of a prostatic carcinoma cell line,[6] inhibit the growth of fibroblasts and their differentiation into adipocytes (perhaps the reason for its purported benefits in obesity)[7] protect against acute lethal viral infections,[8] inhibit the hepatic glucose-6-phosphatase system,[9] enhance interleukin-2 synthesis by helper T cells,[10] augment oxidant injury by inhibiting rabbit lung glucose-6-phosphate dehydrogenase,[11] and demonstrate antiglucocorticoid action.[12] Therefore there are a number of both potentially beneficial and detrimental effects of this steroid, formerly believed to be largely inactive.

To obviate some of the potential deleterious effects of the steroid, the authors have developed a novel lipophilic matrix; they postulate that lipophilic vehicle will allow the DHEA to bypass the liver and enter the lymphatics.

The authors have administered single doses of micronized DHEA (150 and 300 mg) to eight postmenopausal women in a randomized fashion. They have studied the increases in DHEA, DHEAS, testosterone, and estradiol for 72 hours and compared the results with those of the same group of women receiving placebos. Significant increases in DHEA and DHEAS values over control levels were reached with both doses, with mean peak values of DHEAS seen at approximately 240 to 300 minutes and those of DHEA at approximately 100 minutes. A dose-dependent effect was noted at multiple time points for DHEAS and at the time of peak DHEA concentrations. Serum testosterone also increased significantly after the short-term oral administration of DHEA and remained elevated 720 minutes after administration. No significant change in estradiol was observed. It would have been of interest to measure estrone as well, because aromatization of androstenedione, a more proximate product of DHEA bioconversion, is primarily to estrone.

The study is relatively straightforward. The administration of oral micronized DHEA produced the expected rises in DHEA and DHEAS. Single-dose administration is an appropriate prelude to longer-term dosing.

However, several cautions and questions should be raised. First, although the vehicle is different from those previously and one that the authors postulate should not be associated with deleterious effects of DHEA administered in a nonlipophilic matrix, this remains to be determined. The increase in insulin resistance, decrease in sex hormone–binding globulin, and increase in potent circulating and locally formed androgens are concerns. Further, the authors state that postmenopausal women would "accrue benefits of premenopausal DHEA concentrations without the deleterious effects from an excess of potent androgen metabolites." Unfortunately, however, they have not measured androgen metabolites other than testosterone.

Additionally, finding elevated liver function studies and hepatitis in one of the eight women after single-dose administration is also a concern, particularly when long-term use is contemplated. This could, of course, just have been a chance event.

Because the authors found that the assay used for quantitation of testosterone in unextracted serum overestimated true testosterone values by 300%, it would have been preferable if all samples had been reassayed with an extraction procedure rather than arbitrarily reducing the levels by a third. The authors did, however, demonstrate a linear correlation between unextracted and extracted values.

Further, the authors state that pharmacologic doses of DHEA exert "salutary effects on serum lipoprotein profiles." How should this statement be interpreted in view of the known deleterious effects on liver lipoprotein information caused by chronic administration of androgenic compounds?

The authors state that there was only a "comparatively modest" rise in testosterone. However, testosterone is a much more potent androgen than DHEA or DHEAS. Therefore even a modest rise should not be ignored.

The authors have carried out a careful study with a novel lipophilic matrix for what may be a useful com-

pound. The data are clearly presented and analyzed and the possible shortcomings and some of the potentials risks noted. Obviously a good deal more work needs to be performed before the long-term efficacy and safety of this steroid can be determined.

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Dr. Robert A. Wild, Oklahoma. What was the effect on hot flashes?

Dr. Buster (Closing). In response to the first question, we measured estrone, and the values were <30 pg/ml. There was no increase in the concentrations of estrone after DHEA. We did the measurements after we submitted the manuscript, and that is why you did not see it in the paper.

The second question has to do with the vehicle, the DHEA dose, and the effects it produces. The lipid matrix vehicle allows us to partially bypass the liver. We hoped it would change the profile of metabolites. Of particular concern is the conversion of oral DHEA to testosterone and its effect on insulin resistance. We hoped that with the lipid matrix the absorption by the lymphatic system would allow some bypassing of the liver. We were reassured when we did not see as much testosterone conversion as had been the case in previous studies with crystalline steroid.

The critical issue, though, was not with the vehicle but with the DHEA dose. The doses used were actually the lowest that we have seen. The end point we were seeking was a dose low enough to keep the testosterone within a physiologic range. The idea was to avoid some of the problems of high testosterone that had been seen previously.

The underlying purpose of the study was to find a dose of DHEA suitable for replacement. We looked at the testosterone level as one of several critical end points required to design the multiple-dose study that we plan to do next. In that study we will seek to avoid producing the insulin resistance effects that were seen in the previous studies.

The last issue that Dr. Jaffe mentioned was that of lipoproteins. In looking at the literature, we found that reports are at variance as to the effect of DHEA on lipoproteins. Existing studies uniformly show a decrease in cholesterol, but the ratio of high density and low density varies between the studies. The animal data are similarly confusing. We hope that with lower doses and with lipid matrix, the larger portion of DHEA that bypasses the liver will allow the liver to produce the beneficial metabolites without excess testosterone. This issue has to be looked at very carefully before beginning additional studies on a large scale.

We certainly do have more work planned with replacement DHEA. A long-term repeat dose study is being designed; its purpose is to analyze the biologic effects of DHEA replacement. We hope that with lower DHEA doses the problems with the virilizing effects of testosterone we had observed previously will not continue. At the same time we hope that things that seem to be good about DHEA can still be obtained. I imagine that like everything else there are many counterbalancing effects, both good and deleterious, that we have to look for. More work is planned.

Regarding Dr. Wild's question about hot flashes, of course the women on the study were off estrogen. One of the women who took the drug began having hot flashes as the dose cleared from her circulation. A curious observation.

Endothelium-derived relaxing factor and indomethacin-sensitive contracting factor alter arterial contractile responses to thromboxane during pregnancy

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OBJECTIVES: Pregnancy reduces uterine artery contractile responses to norepinephrine and angiotensin II in many species, including the human and the guinea pig, by release of endothelium-derivec relaxing substances. We hypothesized that vascular reactivity to thromboxane during pregnancy would also be reduced by a similar mechanism.

STUDY DESIGN: Isolated ring segments of uterine and carotid arteries from nonpregnant and near-term pregnant guinea pigs were suspended in a myograph for the measurement of isometric tension.

RESULTS: Uterine but not carotid artery sensitivity to cumulative addition of the thromboxane analog U46619 was decreased during pregnancy. The maximal contractile responses of both vessels were unaltered by pregnancy. Nω-nitro-L-arginine (10^{-4} mol/L), an inhibitor of nitric oxide endothelium-derived relaxing factor synthesis, increased the sensitivity of uterine and carotid arteries to U46619 in both pregnant and nonpregnant animals. The maximal contractile response of uterine arteries from pregnant guinea pigs was also increased, but that of nonpregnant ones was not. The maximal U46619 contractile response of the carotid artery was not significantly altered by Nω-nitro-L-arginine. Indomethacin (10^{-5} mol/L), a cyclooxygenase inhibitor, reduced both the sensitivity and the maximal response of U46619 in each vessel group. Removal of the endothelium from uterine artery of pregnant animals enhanced both sensitivity and maximal response to U46619. Pretreatment of the denuded segments with indomethacin reduced the sensitivity to U46619. However, indomethacin-treated denuded segments were still more sensitive to U46619 than controls.

CONCLUSION: The sensitivity of guinea pig uterine artery but not carotid artery to thromboxane is reduced during pregnancy. Although the precise mechanism remains unclear, both endothelium-derived relaxing factor and an indomethacin-sensitive contracting factor are involved. If indomethacin-sensitive contracting factor is released by humans and disease alters that release, it is possible that any enhanced contractile response to thromboxane resulting from the loss of endothelium-derived relaxing agents such as prostacyclin and endothelium-derived relaxing factor would be offset by the loss of indomethacin-sensitive contracting factor. (Am J Obstet Gynecol 1992;166:1171-81.)

Key words: Pregnancy, thromboxane, U46619, endothelium-derived relaxing factor, prostacyclin, Nω-nitro-L-arginine, indomethacin, uterine artery, carotid artery, guinea pig

Pregnancy profoundly alters blood flow. For example, flow through the uterine artery of the guinea pig increases 3500% above that of the nonpregnant animal.[1] Although it is clear that the redistribution of an increased cardiac output to certain organs is crucial for a successful pregnancy, the mechanisms by which this redistribution occurs are poorly understood. It is associated with decreased uterine artery contractile responses to norepinephrine and angiotensin II[2, 3] coupled with increased prostacyclin[4, 5] and nitric oxide endothelium-derived relaxing factor (EDRF).[6, 7] The latter is synthesized from L-arginine by nitric oxide synthetase. Increased prostacyclin contributes to the reduction in uterine artery sensitivity to angiotensin II,[5] whereas the decreased contractile responses of uterine artery to norepinephrine result directly from enhanced nitric oxide EDRF.[8]

Pregnancy also has a profound effect on coagulation. There is enhanced but, for the most part, balanced consumption of both soluble clotting and platelet components.[9] This balance is lost in women with preeclampsia and in women whose fetuses are growth restricted

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because of uteroplacental insufficiency. In each the endothelium appears dysfunctional[10-14] and both the thromboxane/prostacyclin ratio and the consumption of is platelets increased.[15-19] Several investigators have hypothesized that the platelet-released thromboxane in these disorders is the cause of the increased systemic or placental resistance or blood pressure.[17-19] In support of this hypothesis, aspirin at a dose that reduces both platelet aggregability and circulating thromboxane but does not significantly affect endothelial prostacyclin synthesis[20] decreases the incidence of preeclampsia[21] and improves fetal growth in pregnancies complicated by uteroplacental dysfunction.[22] Thus it is important to understand the effect of pregnancy on vascular responses to thromboxane.

A few studies have examined the effect of thromboxane on the uterine circulation. Uterine ischemia in the dog causes hypertension as a result of thromboxane released by the uterine vascular bed.[23] The infusion of a stable thromboxane analog, U46619, constricts the ovine fetal-placental vasculature. The constriction is not reversed by prostacyclin.[24] Neither the effect of pregnancy on contractile responses to U46619 nor the endothelium-derived factors that might modulate it have been investigated. We hypothesized that, like norepinephrine and angiotensin II, the contractile response of vascular smooth muscle to U46619 would be decreased during normal pregnancy and that EDRF or prostacyclin would play a role in that reduction. Because both release and action of these potential modulators are local, we tested this hypothesis with isolated uterine and carotid arteries from pregnant and nonpregnant guinea pigs.

Material and methods

The protocols used for this investigation were approved by the University of Iowa Animal Care Committee.

Preparation: Suspended arterial rings. Adult female nonpregnant and pregnant (50 to 60 days' gestation, term 65 to 68 days) mixed breed guinea pigs were anesthetized (ketamine hydrochloride 80 mg/kg intraperitoneally, xylazine 3 mg/kg intramuscularly). The common carotid and main uterine arteries were removed en bloc with microsurgical techniques as described previously.[2, 7] The uterine artery was selected because of its dramatic increase in blood flow during pregnancy[1] and our prior demonstration of decreased norepinephrine contractile responses with enhanced EDRF.[8] The carotid artery was selected because contractile responses to norepinephrine were unaffected by pregnancy.[8] The vessels were placed into iced Krebs-Ringer solution composed of sodium chloride 118 mmol/L, calcium chloride 2.2 mmol/L, potassium chloride 4.7 mmol/L, $MgSO_4 7HOH$ 1.2 mmol/L, potassium orthophosphate 1.21 mmol/L, sodium bicarbonate 25 mmol/L, and glucose 11.1 mmol/L. They were cleaned of loose connective tissue, cut into 3 mm rings free of side branches, and suspended on two 50 μ tungsten wire triangles. One triangle was hooked to a stationary rod and the other to a strain gauge transducer (Grass FT 0.03, Quincy, Mass.) for the measurement of isometric force. The rings were suspended in 25 ml chambers filled with Krebs-Ringer solution maintained at 37° C and aerated with 95% oxygen and 5% carbon dioxide. The pH of the baths was maintained between 7.38 and 7.45.

After 60 minutes, the rings were stretched incrementally to their individual optimal resting tensions. Optimal resting tension was determined by the contractile responses to 40 mmol/L potassium chloride. Subsequently, the rings were constricted (with either prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (5×10^{-6} mol/L) or phenylephrine (10^{-4} mol/L)) to 70% to 80% of maximal response and challenged with acetylcholine (10^{-4} mol/L). Relaxation confirmed the presence of functionally intact endothelium.[7] Rings unresponsive to acetylcholine were discarded.

At the conclusion of an experiment, the ring was opened longitudinally, and the circumference (millimeters) and weight (milligrams) were recorded after the vessel was first lightly blotted dry with a paper towel. The cross-sectional area (square millimeters) was calculated by:

$$\text{Area} = 2 \times (\text{Wet weight}/1.06) \times \text{Circumference}$$

where 1.06 represents an estimate of tissue density (milligrams per cubic millimeter). Isometric force was normalized for the cross-sectional area of each tissue segment.[25]

Experiments. To determine whether pregnancy alters vascular responsiveness to the cumulative addition of U46619 (10^{-10} to 10^{-5} mol/L), the contractile responses were measured in suspended uterine and carotid arteries from pregnant and nonpregnant guinea pigs.

To determine whether EDRF modulates the vascular response to U46619, contractile responses were measured in suspended uterine and carotid arteries pretreated with a potent inhibitor of nitric oxide EDRF synthesis—Nω-nitro-L-arginine (10^{-4} mol/L for 30 minutes before the experiment).[26]

To determine whether cyclooxygenase products modulate the contractile response to U46619, contractile responses were measured in suspended uterine and carotid rings pretreated with indomethacin (10^{-5} mol/L for 30 minutes before and during the experiment).

To determine whether the endothelium was the site of the pregnancy-associated changes, uterine artery

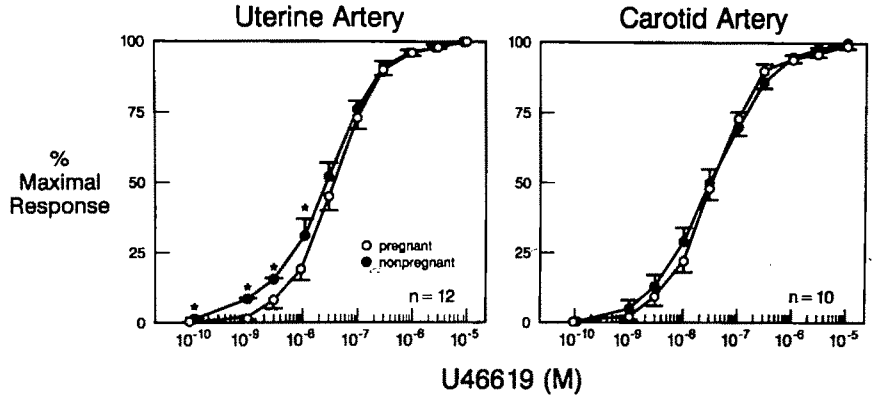


Fig. 1. Effect of pregnancy on uterine *(left panel)* and carotid *(right panel)* arteries sensitivity to cumulative doses of thromboxane analog U46619. Pregnant *(open circle)*, $n = 12$; nonpregnant *(closed circle)*, $n = 10$. There is a significant decrease in sensitivity of uterine artery from pregnant animals to U46619. Carotid artery sensitivity was unaltered by pregnancy. *Asterisk*, Significant difference at that dose by unpaired t test ($p < 0.05$). *M*, mol/L.

segments from pregnant animals were mechanically denuded by passing a 4-0 silk suture through the lumen. Destruction of the endothelium was confirmed by the loss of acetylcholine-stimulated relaxation. Subsequently, cumulative responses to U46619 were measured in the absence and presence of indomethacin.

Control segments for each experiment were run in parallel. All experiments were performed in the presence of desipramine (10^{-5} mol/L), hydrocortisone (10^{-5} mol/L), and propranolol (10^{-5} mol/L) to block neuronal and extraneuronal uptake of norepinephrine and β-adrenergic receptors, respectively.[27, 28]

Drugs. The following drugs were used: acetylcholine hydrochloride, desipramine, hydrocortisone, indomethacin, propranolol, U46619, and Nω-nitro-L-arginine (Sigma Chemical Co., St. Louis), $PGF_{2\alpha}$ (Upjohn Pharmaceuticals, Kalamazoo, Mich.), ketamine hydrochloride (Bristol Meyers Co., Syracuse, N.Y.), and xylazine (Miles Laboratories, Shawnee, Kan.). All drugs and buffers were prepared immediately before the experiment and stored on ice until used. Concentrations are expressed as the final molar concentration in the vessel chamber.

Analyses. The data are expressed either as the mean ± SEM or as the percent of the maximal response to the agonist U46619 in the experiment; n refers to the number of animals. Inhibition of acetylcholine-stimulated relaxation by Nω-nitro-L-arginine and its effect on $PGF_{2\alpha}$-induced tone were tested with paired and unpaired t tests. Dose-response analyses used a two- or three-way random block repeated-measures analysis of variance. Either contractile force and the percent maximal response were the dependent variables and dose, pregnancy status, and treatment the independent variables (blocking by animal). If the mean values for the analysis of variance were found to be significantly different ($p \leq 0.05$), either a Scheffé or t test was applied. Thus, in spite of large standard errors caused by interanimal variability, statistically significant differences among independent variables could be discerned.

Results

Effect of pregnancy on contractile responses to U46619. Significant dose-dependent contractile responses to U46619 were documented in each experimental sequence. The sensitivity to U46619 was significantly reduced in uterine (but not carotid) arteries from pregnant animals compared with nonpregnant ones (Fig. 1). Pregnancy had no effect on the maximal response of uterine and carotid arteries to U46619 (not shown).

The maximal contractile response for each ring to potassium chloride (120 mmol/L) was determined at the conclusion of some experiments. Pregnancy enhanced potassium chloride–induced contractions of uterine arteries (676 ± 87 vs 348 ± 99 mg/mm², $p = 0.01$, $n = 16$), whereas the response of the carotid artery was not unaltered (2502 ± 361 vs 2127 ± 222 mg/mm², $p = 0.21$, $n = 16$). Thus the effect of pregnancy on smooth muscle contractile responses varied with the agonist. If the contractile responses were normalized to the 120 mmol/L potassium chloride response, the maximal contractile response of uterine arteries to U46619 would be reduced during pregnancy. Acetylcholine-stimulated relaxation of preconstricted control uterine artery segments was increased by pregnancy (56% ± 6% vs 29% ± 3%, $p = 0.0006$).

Effect of EDRF inhibition on U46619 contractile responses. Nω-nitro-L-arginine reduced acetylcholine-stimulated relaxation of preconstricted uterine and carotid arteries regardless of pregnancy status (Table I).

Table I. Effect of Nω-nitro-L-arginine on $PGF_{2\alpha}$-stimulated arterial contraction and acetylcholine-stimulated relaxation (n = 8)

	Uterine artery		*Carotid artery*	
	Pregnant	*Nonpregnant*	*Pregnant*	*Nonpregnant*
Acetylcholine (percent relaxation)				
Control	48 ± 10	24 ± 2*	84 ± 4	88 ± 4
Nω-nitro-L-arginine	24 ± 9*	5 ± 2†	40 ± 4†	37 ± 7†
$PGF_{2\alpha}$ (mg/mm²)				
Control	821 ± 151	840 ± 175	501 ± 54	360 ± 75
N-ω-nitro-L-arginine	1116 ± 146†	1100 ± 274†	751 ± 66†	527 ± 101†

Values are mean ± SEM.
*$p < 0.05$, from pregnant.
†$p < 0.05$, from control.

Nω-nitro-L-arginine enhanced $PGF_{2\alpha}$ tone in the uterine and carotid arteries (Table I). The mean percent increase in the contractile response of uterine artery to 5×10^{-6} mol/L $PGF_{2\alpha}$ from pregnant animals compared with nonpregnant animals did not reach significance in this series (44% ± 11% vs 27% ± 12%, respectively, p = not significant). The maximal contractile response to 120 mmol/L potassium chloride was unaltered by Nω-nitro-L-arginine in either vessel (not shown).

Nω-nitro-L-arginine enhanced the maximal contractile response to U46619 of uterine arteries obtained from pregnant animals compared with nonpregnant ones (1590 ± 161 vs 1879 ± 111 mg/mm², $p < 0.05$) (Fig. 2). The contractile response of uterine artery from nonpregnant animals to U46619 was not significantly altered by Nω-nitro-L-arginine. The effect of Nω-nitro-L-arginine appeared greatest at the upper end of the U46619 dose-response curve. Nω-nitro-L-arginine also enhanced the sensitivity of uterine and carotid arteries from pregnant animals and carotid artery from nonpregnant animals to U46619 compared with untreated controls (not shown). Thus nitric oxide EDRF blockade had the least effect on the vessel with the smallest baseline relaxation response to acetylcholine—uterine artery from nonpregnant animals.

Effect of indomethacin on U46619 contractile responses. Neither the acetylcholine-induced relaxation nor the phenylephrine-induced contraction from which that relaxation was measured was altered by indomethacin.

The effect of indomethacin on contractile responses to U46619 was not pregnancy dependent. Indomethacin reduced the sensitivity of both uterine and carotid arteries to U46619 (Fig. 3). The effect of indomethacin on the dose-response curve was most noticeable on the lower end. Because the reduction in uterine artery sensitivity during pregnancy to U46619 also occurred at the lower end of the dose-response curve, contractile responses of uterine artery to U46619 no longer differed between pregnant and nonpregnant animals after indomethacin (not shown). Perhaps because of the wide interanimal variation in contractile responses to high concentrations of U46619, the maximal contractile response to U46619 was significantly reduced by indomethacin only in the carotid arteries obtained from pregnant animals (Fig. 4).

Effect of endothelial destruction on U46619 contractile responses. Removal of the endothelium from uterine arteries of pregnant animals abolished the acetylcholine-induced relaxation and enhanced their sensitivity to U46619 (Fig. 5). Treatment of the denuded vessels with indomethacin reduced their sensitivity to U46619. However, the sensitivity of indomethacin-treated denuded vessels was still greater than control. The rise in the maximal contractile response to U46619 after denudation did not reach significance (1321 ± 95 versus 1766 ± 220 mg/mm², p = not significant).

Comment

In the nonpregnant animal and human, platelet aggregation and the response of vascular smooth muscle to contractile agents are modulated by the release of endothelium-derived substances such as nitric oxide EDRF and prostacyclin.[29] During the human and guinea pig pregnancy, acetylcholine-stimulated nitric oxide EDRF relaxation of the uterine artery is increased.[6, 7] Sheep uterine artery synthesis of prostacyclin during pregnancy is also increased.[4] Recently we demonstrated that the decreased contractile responses of uterine artery to norepinephrine that are characteristic of pregnancy result directly from enhanced nitric oxide EDRF release.[8] Previously, Magness[5] observed that the decreased sensitivity of uterine artery to angiotensin II during pregnancy is secondary to enhanced release of endothelium-derived prostacyclin. These findings lead us to postulate that the enhanced release of endothelium-derived dilator substances during pregnancy would also reduce the contractile responses of uterine artery to thromboxane. If any re-

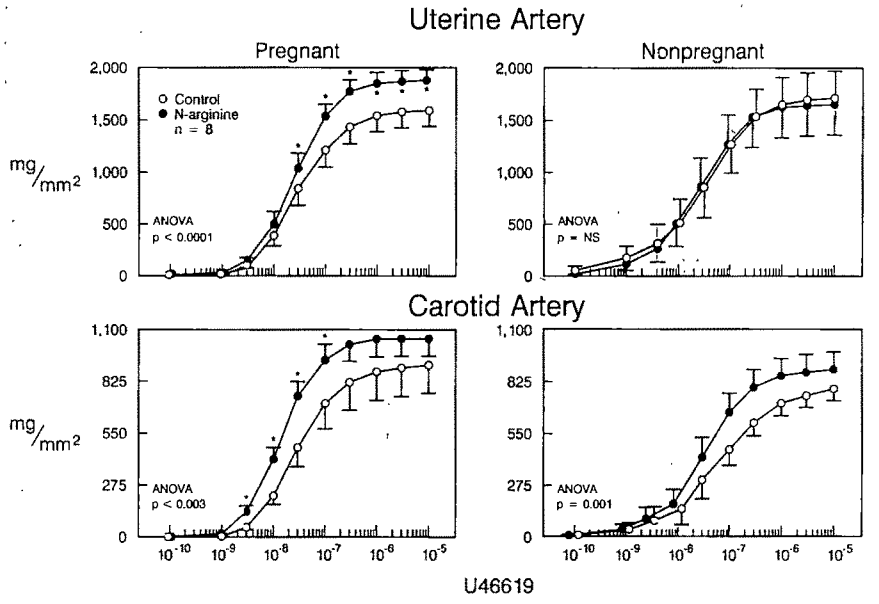


Fig. 2. Effect of Nω-nitro-L-arginine *(N-arginine)* on cumulative contractile responses of uterine *(upper two panels)* and carotid arteries *(lower two panels)* to U46619. Pregnant *(left panels)*, $n = 8$; nonpregnant *(right panels)*, $n = 8$. Maximum contractile response of uterine artery obtained from pregnant animals was enhanced significantly compared with control *(open circles)*. *Asterisk*, Significant difference at that dose by paired t test ($p < 0.05$). Analysis of variance *(ANOVA)* refers to comparison of two curves.

duction in sensitivity to thromboxane was dependent on enhanced tonic or stimulated release of these endothelium-derived dilators, then a pregnancy disorder characterized by increased release of platelet-derived thromboxane could result in vasospasm. Because these endothelium-derived modulators are locally acting substances, we examined then in vitro to eliminate the uncontrollable variables inherent to the in vivo study of contractile agents.

We found that the sensitivity of uterine but not carotid artery to U46619 was reduced during pregnancy. Pregnancy increased the maximum potassium chloride–induced contractile response by a mechanism currently under investigation. When the responses to U46619 were normalized to the potassium chloride maximum, there was a right shift of the dose-response curve during pregnancy. This suggests that either thromboxane receptor number or affinity is reduced during pregnancy or that modulators of the smooth muscle response to thromboxane are altered. Although not examined in this study, gender-dependent differences in prostaglandin receptors have been reported.[30]

To determine the role of nitric oxide EDRF in modulating the contractile response to thromboxane during pregnancy, we inhibited its synthesis from L-arginine by nitric oxide synthetase[31] with Nω-nitro-L-arginine.[26] Stimulated release of nitric oxide EDRF by acetylcholine as evidenced by increased relaxation was significantly greater in uterine artery from pregnant animals than from nonpregnant ones. Nω-nitro-L-arginine enhanced the response of uterine and carotid arteries to $PGF_{2\alpha}$ and inhibited acetylcholine-stimulated nitric oxide EDRF relaxation in both uterine and carotid arteries. In prior studies we found that the contractile response to $PGF_{2\alpha}$ was enhanced significantly more in uterine arteries from pregnant animals than from nonpregnant ones.[18] The current study supports that observation. This suggests that tonic release of nitric oxide EDRF from uterine artery is also increased during pregnancy. Nitric oxide EDRF blockade by Nω-nitro-L-arginine enhanced the sensitivity of uterine and carotid arteries to U46619 regardless of pregnancy status. Nitric oxide EDRF blockade did not affect U46619 contractile responses of uterine arteries from nonpregnant animals. This suggests that the modulation of U46619 contractile responses in the uterine artery by either tonic or stimulated release of nitric oxide EDRF is specific for pregnancy. The findings also suggest that nitric oxide EDRF modulates the U46619 contractile response of carotid artery regardless of pregnancy status.

To determine the potential effect of cyclooxygenase products on contractile responses to U46619, we inhibited the pathway with indomethacin. We selected indomethacin because it increases the systemic pressor response of pregnant humans and a variety of animals to angiotensin II.[32, 33] We found that indomethacin also alters the contractile responses of both uterine and carotid artery to U46619 but decreased rather than in-

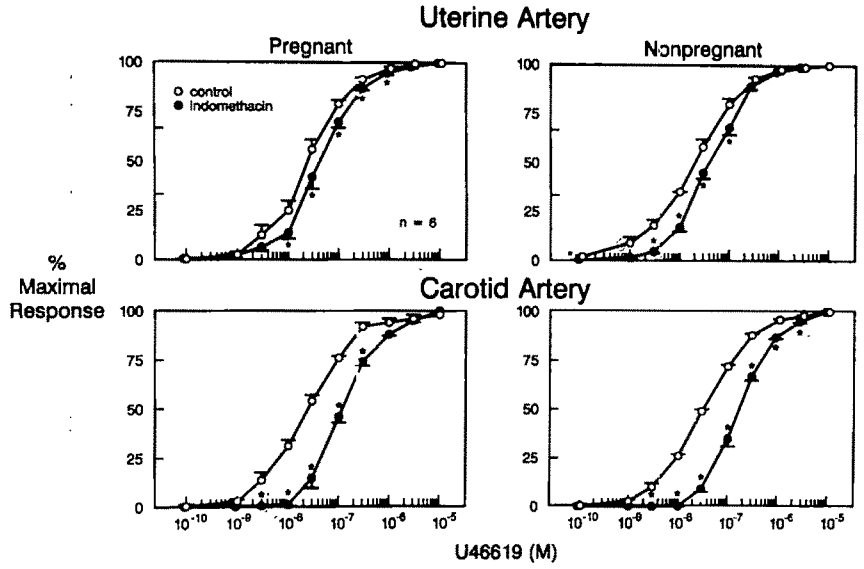


Fig. 3. Effect of indomethacin on uterine *(upper panels)* and carotid *(lower panels)* artery sensitivity to cumulative doses of U46619. Pregnant *(left panels)*, $n = 6$; nonpregnant *(right panels)*, $n = 6$. Sensitivity was significantly diminished by indomethacin *(closed circles)* in each vessel group compared with control *(open circles)*. *Asterisk*, Significant difference at that dose by paired t test ($p < 0.05$).

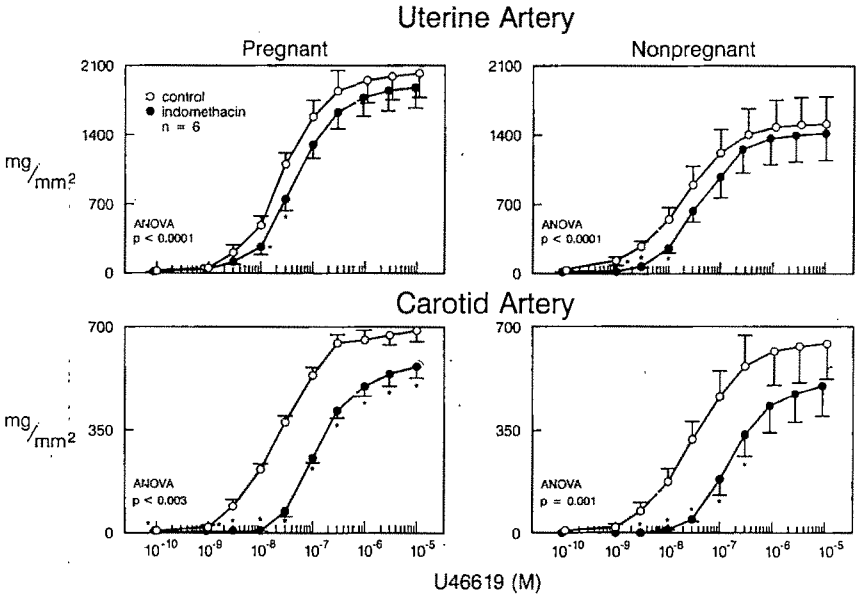


Fig. 4. Effect of indomethacin on contractile responses to cumulative doses of U46619 in uterine artery *(upper panels)* and carotid artery *(lower panels)* from nonpregnant *(left panels)* and pregnant *(right panels)*. Indomethacin *(closed circles)* caused significant right shift in contractile responses to U46619 compared with control *(open circles)*. *Asterisk*, Significant difference at that dose by paired t test ($p < 0.05$). Analysis of variance *(ANOVA)* refers to comparison of two curves.

creased sensitivity as had been expected. Indomethacin affected uterine and carotid artery contractile responses to U46619 regardless of pregnancy status. The percent decrease in contractility after indomethacin was much greater in the carotid artery than in the uterine artery. This suggests that U46619 stimulates the release of an indomethacin-sensitive contracting factor from both uterine and carotid arteries. However, indomethacin-sensitive contracting factor has a greater role in modulating carotid artery response to U46619 as compared with uterine artery response.

Although both nitric oxide EDRF blockade and in-

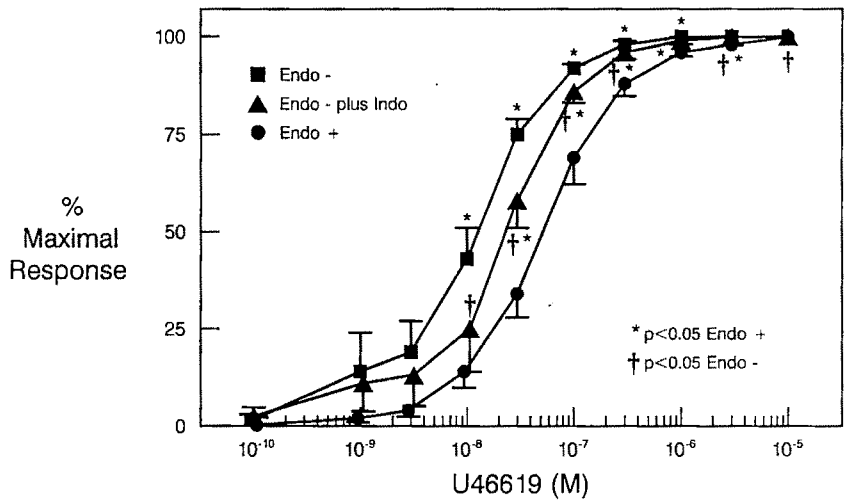


Fig. 5. Effect of mechanical removal of endothelium in uterine artery from pregnant animals, $n = 7$. Denudation *(closed square)* enhanced contractile responses to U46619 compared with control *(closed circle)*. Addition of indomethacin to denuded segments *(closed triangles)* decreased sensitivity to U46619 compared with that of denuded segments. However, contractile responses were still greater than control. *Asterisk*, $p < 0.05$, compared with endothelium-intact control; *dagger*, $p < 0.05$, compared with endothelium-denuded segments.

domethacin altered the U46619 dose-response curve, their principal impact was on different portions of the curve. The effect of nitric oxide EDRF blockade was greatest on the upper portion of the dose-response curve, whereas the effect of indomethacin was greatest on the lower portion. This suggests that more than one mechanism modulates the uterine artery response to thromboxane. Because (1) the effect of indomethacin was on the same portion of the curve altered by pregnancy and (2) the responses of uterine arteries from pregnant and nonpregnant animals were similar after indomethacin, we would speculate that pregnancy decreases an indomethacin-sensitive contracting factor, which could account for reduced vascular reactivity to U46619.

Removal of the endothelium from uterine arteries of pregnant animals enhanced the sensitivity to U46619. This confirms that EDRF release by the uterine artery modulates the contractile response to U46619. The addition of indomethacin to denuded rings reduced the sensitivity to U46619, although they remained significantly more sensitive to U46619 than did the control rings. This demonstrates that at least some of the indomethacin-sensitive contracting factor released by the uterine artery is produced by the smooth muscle.

Although this is the first investigation into the effect of pregnancy on contractile responses to thromboxane and the factors that might modulate it, the responses of pulmonary artery to U46619 have been studied in male and nonpregnant female guinea pigs.[34] The response of the pulmonary artery was different from that observed for the uterine artery. Removal of the pulmonary artery endothelium reduced the maximal contractile response to U46619 only in vessels obtained from female animals and not in those obtained from male animals. We have previously found that the contractile responses of carotid arteries to U46619 from female guinea pigs were reduced by indomethacin, but the responses of those vessels from the male were unaltered (Weiner CP, et al. Unpublished observations). These findings suggest that the site of synthesis and release of indomethacin-sensitive contracting factor can vary among vascular beds and that it is a characteristic of the female vasculature.

Preeclampsia and some types of idiopathic growth retardation are associated with a reduction in prostacyclin synthesis.[14, 17-19] Coupled with other markers of endothelial health, both of these disorders are also associated with dysfunctional endothelium,[10-18] culminating in an altered prostacyclin/thromboxane ratio and enhanced platelet consumption.[35] We would speculate that nitric oxide EDRF synthesis or release, like prostacyclin, is also reduced in pregnancies complicated by these disorders. If the response of the human is similar to that of the guinea pig, as it is for the effect of pregnancy on norepinephrine/angiotensin II–stimulated constriction and acetylcholine-stimulated nitric oxide EDRF relaxation, any reduction in endothelium-derived relaxing substances might be offset by a concurrent reduction in indomethacin-sensitive contracting factor. As a result, smooth muscle response to thromboxane could remain the same or decrease rather than increase as has been suggested.[17-19]

In conclusion, the contractile responses of uterine

but not carotid artery to thromboxane are reduced during pregnancy. The effect of EDRF blockade on uterine contractile responses to thromboxane are specific for pregnancy. Thromboxane stimulates the release of an indomethacin-sensitive contracting factor from both uterine and carotid arteries. The contracting factor plays a greater role in modulating the carotid artery responses to thromboxane than it does in the uterine artery. Each endothelial factor is important, but neither alone definitively accounts for the effect of pregnancy on uterine artery sensitivity to thromboxane. If this contracting factor is also released by women, it is possible that the loss or reduction of the endothelium-derived relaxing substances would be offset by the loss of contracting factor and the vascular response to thromboxane would remain unchanged.

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Discussion

Dr. Valerie M. Parisi, Houston, Texas. The maternal physiologic adaptation to normal pregnancy is characterized by marked alterations in uterine perfusion and systemic vascular function. As shown in this schematic representation, the resultant relaxant effect on arteriolar tone is achieved through a complex and, as yet, an incompletely understood interaction of many vasoactive substances.

It is thought that increased endothelial production of vasodilators such as prostacyclin and EDRF during pregnancy is primarily responsible for the observed loss of vascular responsiveness to otherwise potent vasoconstrictors, such as norepinephrine and angiotensin II.

Pregnancies complicated by preeclampsia present a vascular physiologic disorder marked by an imbalance in eicosanoid production that favors vasoconstriction. Compared with control placentas, placentas from women with preeclampsia show a marked decrease in prostacyclin production coupled with increased thromboxane production.[1] Biochemical alterations in both trophoblast and placental vascular endothelium contribute to this imbalance.

Recently, investigation in this arena has focused on events occurring at the cellular and subcellular levels to further elucidate mechanisms of vascular control.

Dr. Weiner and his colleagues have elegantly studied the contractile responses of uterine and carotid arteries to the thromboxane analog U46619 during pregnancy and evaluated the role of the vasodilators prostacyclin and EDRF in these responses.

Dr. Weiner's article presents new and exciting information, as neither the effect of pregnancy on vascular responses to thromboxane nor the potential mechanisms by which it occurs have been previously studied. Their work also has enormous clinical relevance, as elucidation of mechanisms by which thromboxane acts, and subsequently may be inhibited in the pregnant vasculature, is of the utmost importance if we are to be successful in the prevention or, if necessary, the treatment of preeclampsia and other placental vascular disorders.

Dr. Weiner hypothesized that the contractile responses of vascular smooth muscle to thromboxane analog would be decreased during pregnancy. This was true for uterine but not carotid arteries. In view of the enhanced response to potassium chloride–induced contractions and the increased acetylcholine-stimulated relaxation of preconstricted uterine artery segments, is it possible that increases in uterine vascular surface area during pregnancy play a role by quantitatively increasing vasodilator production?

The authors report that EDRF inhibition by Nω-nitro-L-arginine modulated pregnant uterine artery responses to thromboxane, although curiously the effect was greatest at the upper end of the dose-response curve. Does the potential quantitative increase in thromboxane production in human disease states approach the concentrations used at this end of the curve?

Indomethacin was used to block the cyclooxygenase pathway leading to prostacyclin production and, contrary to their hypothesis, decreased the sensitivity of both uterine and carotid arteries to thromboxane analog. Unlike the effect of EDRF inhibition, indomethacin's effect was most pronounced at the lower end of the dose-response curve. Dr. Weiner attributes these observations to the presence of an indomethacin-sensitive contracting factor that is decreased in pregnancy. An alternative explanation exists. Indomethacin has produced varied and inconsistent changes in uterine perfusion in whole-animal models. Unfortunately, it is not a specific cyclooxygenase blocker but alters the lipooxygenase pathway as well and has been postulated to have intrinsic vasoactive properties.

I would like to ask Dr. Weiner if he has confirmed his observations by using another, more specific cyclooxygenase blocker and if he could speculate on the identity of the indomethacin-sensitive contracting factor.

The final segment of this article reports increased sensitivity of pregnant uterine arteries to thromboxane analog after endothelial ablation, which is then reduced by the addition of indomethacin. These observations indicate a role for uterine artery smooth muscle in the production of vasoactive substances. Others have quantitated uterine artery prostacyclin production and reported 64% derived from endothelium and 36% from vascular smooth muscle.[2] I would like to ask Dr. Weiner if his data support these percentages?

Last, although it is recognized that the majority of the vasoactive substances described in this article act by autocrine and paracrine mechanisms at the local level, we must not lose sight of the necessity for investigation in the in vivo setting. How might increased thromboxane production in human disease states have an impact on the systemic vasculature, and would there be different organ vascular responses to its increased production?

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Dr. James M. Roberts, San Francisco, California. I have two questions. Would you remind us of the findings with other contractile agonists in terms of the role of indomethacin-sensitive contractile agents and EDRF from the work you've done previously?

Second, I think there is an accumulated literature that indicates that EDRF has its greatest effect on precontracted vessels. So it may well be that the results you're seeing aren't necessarily related to the dose of thromboxane but rather to the contractile state of the vessel. I wonder if you have run experiments that would allow you to sort out whether it's just that the high dose of thromboxane and the vessels were contracted or

whether it really is an independent dose-related phenomenon.

Dr. Thomas H. Kirschbaum, Bronx, New York. I wonder if you would comment in general on the relationship between the study of vasoactive agents in uterine artery versus the arteriolar bed further downstream, where changes in vascular resistance actually influence flow. My understanding is that, with respect to some vasoactive agents, differences in response have been reported between these two sites.

Dr. Raul Artal, Los Angeles, California. I would like to ask if you examined other biomechanical parameters to separate acute from chronic effects and elastic from ductile properties, such as the modulus of elasticity strain to rupture, work to rupture, and sample thickness before and after each experiment.

Dr. Weiner (Closing). First, I thank Dr. Parisi for her summary and for stressing the complexity of these interactions. It's clear that there are going to be no silver bullets, no single agent that is responsible for the diseases and the end effects of the diseases we are concerned with.

Dr. Parisi asked about the role of the increased diameter and actual weight of the uterine artery during pregnancy. In the guinea pig it increases almost threefold, and in the prior investigation that we reported in the American Journal of Obstetrics and Gynecology, we tried to quantitate whether there was any change in the number of endothelial cells present in the vessel that would account for the increased EDRF-mediated relaxation.

We found that the number of cells per unit area was unchanged, but because the unit area was increased, the total number of cells had to be increased. Could that in part be responsible for the increase in EDRF-mediated responses we see? Yes, it certainly could be. We also know that after estrogen administration the increased responsiveness to acetylcholine or the increase in EDRF occurs within hours, and I wouldn't be surprised if we hear very shortly that EDRF plays a crucial role in the estrogen-mediated increase in uterine blood flow.

Dr. Parisi also asked about the physiologic concentration of thromboxane. At the site of the platelet plug, 10^{-6} or 10^{-7} mol/L is readily achievable. Whether you would see 10^{-4} mol/L or as high as we went here, 10^{-5} mol/L, is not very likely.

However, if you look closely at the dose-response curves, you'll note that, from the standpoint of the indomethacin-sensitive contracting factor and also the nitric oxide related components, there were significant changes seen at 10^{-7} and 10^{-6} mol/L.

Dr. Parisi points out that indomethacin, although a very popular drug in obstetric investigations, is certainly not a very "clean" drug. We used indomethacin initially for this study because of its long track record and because of its documented association with an increase in angiotensin II responsiveness. We've since repeated these studies to a much smaller extent with ibuprofen and found the same responses.

When we identified this decrease in responsiveness to U46619 after pretreatment with indomethacin, we were very puzzled and went back to the literature. We came across a very exciting article in the *American Journal of Physiology,* I believe from 1983. What these investigators did was examine pulmonary arteries from male and female guinea pigs, and they found that, when they removed the endothelium, the response to U46619 increased in the male, and decreased in the female animals.

We have repeated these studies with coronary and carotid arteries from male and female animals and can confirm the finding of these investigators that this indomethacin-sensitive contracting factor is gender specific. It is not in male animals. It does appear to vary, whether it comes from the endothelium or from the smooth muscle by the vascular bed.

We have not specifically identified the contracting factor, although there is good reason to suspect, from some preliminary data and from studies done in alloxan-treated rabbits, that the factor is thromboxane and that the endothelium can, under the appropriate circumstances, synthesize thromboxane into a great concentration. We have not tried to quantitate the factor yet, and that is on our list of things to do in the future.

The final question that Dr. Parisi asked was about the variations of endothelium-derived factors, whether they be EDRF or prostacyclin or endothelium-derived hyperpolarizing factor or any of the agents that are coming to light. Clearly there is a difference among vascular beds—among vascular beds, among the arteries and veins, among hormonally sensitive vascular beds and non–hormonally sensitive beds. It makes a very exciting area of research, to explore not only the effects of pregnancy but also differences in gender by trying to quantify these differences. It holds out the possibility of gender-specific therapies.

Dr. Roberts asked about other contractile agents we and others have explored in looking at the effects in these endothelium-derived relaxing substances on contractile responses in pregnant and nonpregnant animals, and again it demonstrates that there is no silver bullet. The work from the Dallas group very clearly demonstrates, at least in the ewe, that the decreased response to angiotensin II is mediated by prostacyclin and most of that prostacyclin comes from the endothelium.

In contrast, the decreased response to norepinephrine in the guinea pig was solely the result of EDRF. In contrast, work that we presented initially at last year's Society for Gynecologic Investigation meeting, serotonin responses are decreased during pregnancy but appear not to result from either EDRF or prostacyclin. We are exploring that further in our laboratory at this time. Now with thromboxane we see a completely different fourth mechanism.

The second question, if I understood it correctly, that Dr. Roberts had was with whether the stimulated release of EDRF in response to U46619 was a dose-response phenomenon.

Certainly it's harder in the uterine artery because the

standard errors are much larger. In the larger arteries such as the carotid, EDRF inhibition substances produce almost a parallel shift. So I think this response occurs at almost all doses. We've also done some preliminary work looking at the interaction between thromboxane and serotonin in vessels with functional endothelium and nonfunctional endothelium, and as part of that we find that we have to have some active tone in the vessel. The degree of active tone appears to be less important in the interaction so long as there is some active tone there. This leads very nicely into Dr. Kirschbaum's question on the appropriateness of studying conduit vessels. Although the uterine artery in the guinea pig is small, it's still a conduit vessel.

I think in the past people have concluded that the conduit vessels were simply that. They were there to carry the blood to the resistance vessels. We now have some excellent studies suggesting that's not true, and I would direct your attention to a fascinating study that was in the *Journal of Clinical Investigation* about 4 months ago. What these investigators did in vivo was take a rat, open it up, cannulate the renal artery proximally and distally to its entry into the renal bed, and then, with a variety of techniques, either selectively inhibit EDRF or destroy the endothelium; they documented beautifully that the renal artery played a major role in regulating renal blood flow.

I do not know yet that that occurs in the uterine artery. Certainly it is a possibility. It's also quite clear that the uterine artery is responsive to changes in the hormonal milieu; I think it's reasonable at this time to postulate that some of those changes will also occur downstream.

The final question had to do with changes in the uterine artery structure other than those that occurred in response to changes in the endothelium. Clearly that occurs. We have not specifically looked at elasticity, though Griendling et al. looked at the carotid artery in the ewe several years ago and found that there was a very clear difference in the elasticity-to-collagen ratio, but they also found changes in vessel function that they could not attribute simply to changes in elasticity.

If we look at how rapidly, for example, the response to acetylcholine changes in an animal treated with estrogen, just 2 hours, it's long before there's any time for remodeling of vascular smooth muscle. Clearly there is a complex interaction of a number of factors.

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Lp(a) lipoprotein: Relationship to cardiovascular disease risk factors, exercise, and estrogen

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OBJECTIVE: Lp(a) lipoprotein is a distinct lipoprotein particle that recently has been found to be associated with cardiovascular disease. A study was conducted to assess the influence of cardiovascular disease risk factors on levels of Lp(a) and to evaluate the effects of age, exercise, and estrogen on these levels.

STUDY DESIGN: Two studies, a cross-sectional study of older men (n = 105) and women (n = 75) (mean age 76 years) and a prospective study of younger postmenopausal women (mean age 48 years), were carried out. Lp(a) and other lipoproteins were measured in the two studies and differences were sought by statistical analysis.

RESULTS: In the cross-sectional study, serum Lp(a) was similar in men and women and was not influenced by age. Lp(a) levels in men and women were higher when there was more than one cardiovascular disease risk factor present ($p < 0.028$). We could not demonstrate such a relationship with other lipid and lipoprotein measurements. In the prospective study exercise alone had no influence on Lp(a) levels. Oral estrogen decreased Lp(a) levels marginally ($p = 0.08$). The decrease in Lp(a) with oral estrogen was associated with increases in triglycerides ($p < 0.01$) and very-low-density lipoprotein ($p < 0.06$).

CONCLUSIONS: These data confirm that elevated Lp(a) levels are an independent risk factor for cardiovascular disease. Lp(a) levels are primarily influenced by genetic factors and it appears estrogen may have a minor influence on its hepatic synthesis. (Am J Obstet Gynecol 1992;166:1182-90.)

Key words: Lp(a) lipoprotein, cardiovascular disease, estrogen, age, exercise

Nearly 30 years ago a genetic variation of low-density lipoprotein (LDL) that was characterized by an additional protein [apo(a)] on the LDL particle was detected.[1] This altered LDL particle, called Lp(a), is now recognized as a unique, distinct, circulating lipoprotein[2] that contributes to the heterogeneity of LDL. Lp(a) is larger than LDL, has a higher buoyant density, contains its characteristic apolipoprotein (apo B-100), and binds to the LDL receptor.[3] Lp(a) can be dissociated into separate particles of LDL and apo(a) under experimental conditions and in vivo.[3] Apo(a), which is under genetic control, is highly glycosylated and may vary considerably between individuals (200 to 700 kd), thus leading to a large range of values of Lp(a).[4]

Clinical interest in Lp(a) increased when it was realized that elevated levels were associated with atherosclerotic heart disease in both men and women.[5-9] The effects of Lp(a) appear to be independent of the known effects of LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides.[10] The homology between apo(a) and plasminogen[11] has led to the speculation that Lp(a) may also be involved in disease mechanisms where atherogenesis and thrombosis are linked. The heterogeneous nature of Lp(a) and its skewed distribution in white subjects has been explained by variations in the apo(a) gene on chromosome 6, which is within the gene cluster for apo(a) and plasminogen (6q2.6 to q2.7).[12]

Lp(a) therefore appears to be a lipoprotein for which genetic constitution strongly predicts circulating levels. However, there is little information available as to the effects of life-style on levels, especially in women. We evaluated Lp(a) levels in two studies to further examine factors affecting its serum concentration. In the first, a cross-sectional study of older men and women, we evaluated the association between Lp(a) and cardiovascular disease risk factors. In the second we assessed the effects of exercise and estrogen therapy interventions on the Lp(a) levels of young postmenopausal women. Estrogen replacement therapy has been shown to reduce cardiovascular disease mortality.[13] In both studies we compared Lp(a) levels with levels of other lipids and lipoproteins. These two studies together pro-

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Table I. Characteristics of subjects in study 1

	Men	*Women*	*Total*
No.	105 (59.0%)	73 (41.0%)	178 (100%)
Age (yr)*	76.5 ± 0.5	77.2 ± 0.6	76.8 ± 0.4
Medical conditions			
None	44 (41.9%)	45 (61.6%)	89 (50.0%)
At least one	61 (58.1%)	28 (38.4%)	89 (50.0%)
Angina	26 (24.8%)	11 (15.1%)	37 (20.8%)
Heart problem	30 (28.6%)	17 (23.3%)	47 (26.4%)
Myocardial infarction	30 (28.6%)	3 (4.1%)	33 (18.5%)
Stroke	7 (6.7%)	6 (2.7%)	13 (7.3%)
Transient ischemic attack	7 (6.7%)	2 (8.2%)	9 (5.1%)
No. of conditions			
1	33 (31.4%)	19 (26.0%)	52 (29.2%)
2	17 (16.2%)	7 (9.6%)	24 (13.5%)
3	11 (10.5%)	2 (2.7%)	13 (7.3%)

*Mean ± SE.

vide data on the effects of age on the Lp(a) levels of postmenopausal women and provide an assessment of its usefulness as a marker of cardiovascular disease in women.

Material and methods

Study 1

Subjects. Seventy-five women and 105 men were recruited from a cohort of participants in an ongoing study conducted at Leisure World, a retirement community in Laguna Hills, California. The characteristics of this cohort have been described and validated previously.[14] Table I details ages of the individuals recruited for this study and their cardiovascular disease risk factors, which were documented by report and medical records. The subjects ranged in age from 64 to 88 years. Subjects who had a significant history, as diagnosed by a physician, of angina, myocardial infarction, stroke, or transient ischemic attacks were identified from the detailed questionnaire. Subjects with "heart problems" (Table I) included those subjects who had significant heart disease that was not specifically angina or a history of a previous myocardial infarction. Specifically excluded from this cohort were those subjects who were smokers or who had hypertension or diabetes mellitus. None of these subjects had received hormones or lipid-lowering medications in the past.

Protocol. Twenty milliliters of venous blood was obtained with the subject in a fasting state, between 8 and 10 AM. Sera were separated and stored at −20° C until analyzed.

Study 2

Subjects. Sixty-nine women who had undergone hysterectomy or hysterectomy and bilateral salpingo-oophorectomy, who were aged 40 to 55, and who were within 20% of their ideal body weight, were recruited and randomized by computer to one of four groups. Their postmenopausal status had been previously confirmed by elevated follicle-stimulating hormone levels and estradiol levels <25 pg/ml. No woman had any known medical illnesses. No woman had received exogenous hormones for at least 6 months. Subjects were randomized to one of four treatment groups: (1) a 6-month exercise regimen (n = 18), (2) a regimen of 0.625 mg of conjugated equine estrogen (Premarin) taken daily for 6 months (n = 19), (3) a 6-month regimen of conjugated equine estrogen therapy and exercise (n = 16), and (4) a regimen of 0.05 mg of transdermal estrogen (Estraderm) applied twice a week for 6 months (n = 16).

In groups 1 through 4, the ages, heights, and weights were similar as follows: mean ± SE ages 48 ± 1.3, 49.4 ± 1, 48.5 ± 1.3, and 48.1 ± 1.1 years, mean ± SE height 64.4 ± 0.5, 64.6 ± 0.4, 64.9 ± 0.5, and 64.8 ± 0.6 inches, and mean ± SE weight 138.6 ± 7, 142.6 ± 0.5, 144.5 ± 0.8, 143.1 ± 0.7 pounds.

No woman had hypertension or a history of thrombophlebitis, thromboembolic disease, or other cardiovascular disease risk factor. The women also had no current medical illnesses and were not receiving any medications.

For groups 1 and 3, a supervised exercise program was carried out at The Wellness Center, North Florida Regional Medical Center, Gainesville, Florida. This program consisted of walking on a treadmill for 15 minutes followed by pedaling an exercise bicycle for 15 minutes at between 70% and 80% of the maximal heart rate, three to four times per week for the entire 6 months.[15] This exercise regimen was shown to increase $\dot{V}O_2$max by 9% over 6 months.

Fasting blood was obtained from all subjects before enrollment in the study and after 6 months at the end of the regimen. All sera were stored at −20° C until analyzed.

Measurements. Lp(a) in serum was measured by an enzyme-linked immunosorbent assay obtained from

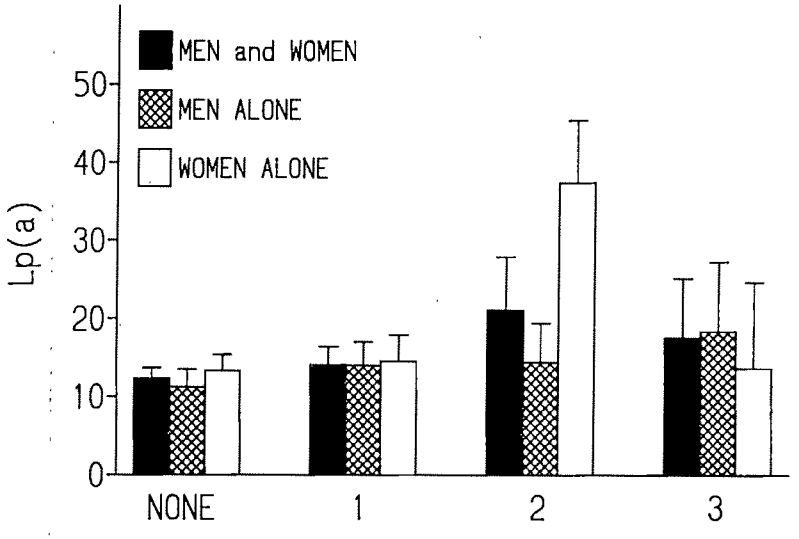


Fig. 1. Mean ± SE Lp(a) levels according to number of medical conditions (none, 1, 2, or 3) in men and women *(solid bars)*, men only *(hatched bars)*, and women *(open bars)*. Results of test for linear trend across categories adjusted for age: men and women, $p = 0.028$; men, $p = 0.103$; women, $p = 0.045$.

Table II. Mean ± SE blood lipids and lipoproteins (mg/dl) of subjects with no cardiovascular disease risk factors in study 1

Lipids	*Men (n = 44)*	*Women (n = 45)*
Age (yr)	75.9 ± 0.8	76.7 ± 0.8
Lp (a)	11.2 ± 1.7	13.3 ± 2.1
Apo A-I	192.0 ± 5.0	208.4 ± 4.3*
Apo B	101.9 ± 4.0	105.4 ± 3.5
Cholesterol	224.7 ± 7.0	254.4 ± 5.8*
Triglycerides	131.0 ± 8.9	148.5 ± 12.9
HDL	31.3 ± 1.6	39.7 ± 2.6*
LDL	168.3 ± 7.1	184.6 ± 6.1*
Cholesterol/HDL	8.2 ± 0.6	7.6 ± 0.5
Apo A-I/apo B	2.0 ± 0.1	2.1 ± 0.1

*Wilcoxon in rank sum test, $p < 0.001$.

Biopool, Hamilton, Ontario, Canada. This assay uses an affinity-purified polyclonal antibody raised against Lp(a) and a human standard obtained from the Blood Bank at Umea University Hospital, Sweden, and is a modification of the method of Guyton et al.[16]

Seventy-five white men and women, aged 22 to 55, were specifically recruited only to determine the precision and reproducibility of the Lp(a) assay in our laboratory. The mean ± SE serum Lp(a) level was 9.75 ± 1.23. The sensitivity of the assay was 0.1 mg/dl, and the intraassay and interassay coefficients of variation were 8% and 12%, respectively. Total cholesterol, triglycerides, and HDL cholesterol were carried out by well-established automated enzymatic procedures.[17-19] The HDL_2 subfraction was measured after precipitation by established methods.[20] LDL cholesterol was calculated with Friedewald's equation. Apo A-I and apo B were measured by immunonephelometric assays. Intraassay and interassay coefficients of variation of these measurements did not exceed 7% and 12%, respectively.

Statistical analyses. For study 1 the Wilcoxon rank-sum test was used to compare blood lipid and lipoprotein levels of men and women and of subjects with and without cardiovascular disease. Subjects were classified according to the number of medical conditions they reported at the time of recruitment into the cohort. A statistical test for linear trend in mean lipid and lipoprotein levels across categories on the basis of these categories was conducted with standard linear regression techniques. In study 2 the association between $\log_{10}$-transformed baseline measurements was assessed with Pearson product-moment correlation coefficients and standard linear regression techniques. The effects of treatment were assessed by repeated-measures analysis of variance. All p values reported are two-sided.

Results

Study 1. Lp(a) and other lipid and lipoprotein measurements were compared in men and women who reported no significant medical conditions (Table II). Serum Lp(a) measurements were similar in men and women, as were levels of apo B and triglycerides. However, total cholesterol, LDL cholesterol, HDL cholesterol, and apo A-I were all significantly higher in women than in men ($p < 0.001$). Ages were similar between the two groups. There was no effect of age on levels of Lp(a) in men or women within the limited age range of the study population. Only apo B correlated

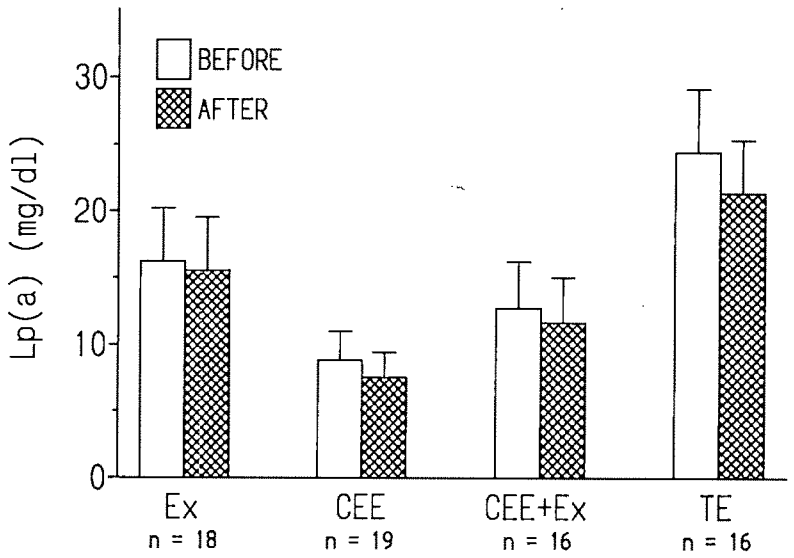


Fig. 2. Mean + SE Lp(a) levels in women before *(open bars)* and after *(hatched bars)* receiving various treatments for 6 months. *Ex*, Exercise; *CEE*, conjugated equine estrogen; *TE*, transdermal estrogen.

Table III. Mean ± SE values of Lp(a) (mg/dl) assessed for all subjects, men and women, with various conditions

Condition	*All*	*Men*	*Women*
None	12.3 ± 1.4 (n = 89)	11.2 ± 1.7 (n = 44)	13.3 ± 2.1 (n = 45)
Angina	16.8 ± 4.8 (n = 37)	12.8 ± 4.0 (n = 26)	26.2 ± 13.0 (n = 11)
Heart problems	13.8 ± 2.4 (n = 47)	13.4 ± 3.1 (n = 30)	14.4 ± 3.9 (n = 17)
Myocardial infarction	18.7 ± 5.6 (n = 33)	15.3 ± 4.4 (n = 30)	53.0 ± 45.6 (n = 3)
Stroke	18.2 ± 5.0 (n = 13)	12.7 ± 4.6 (n = 7)	24.5 ± 9.2 (n = 6)
Transient ischemic attack	35.0 ± 12.3 (n = 9)	39.1 ± 15.1 (n = 7)	21.0 ± 19.0 (n = 2)

negatively with age when men and women were combined ($r = -0.17$, $p < 0.02$) and in the male group alone ($r = -0.19$, $p < 0.05$).

Serum Lp(a) in these older men and women did not correlate with other lipid or lipoprotein parameters, with the exception of LDL ($r = 0.158$, $p = 0.04$).

Measurements of Lp(a) were analyzed on the basis of the medical conditions reported (Table III). In women Lp(a) measurements were higher when the history of angina, heart problems, myocardial infarction, stroke, or transient ischemic attacks was present. However, this relationship failed to achieve statistical significance. When all heart disorders were combined, the values were higher in women with these conditions than in those without disease (19.9 ± 6.0 vs 13.8 ± 2.1 mg/dl), but this result also did not achieve statistical significance.

For transient ischemic attacks, levels of Lp(a) were elevated in men and in women; this elevation was significant for men ($p < 0.05$). Similarly, when stroke and transient ischemic attacks were combined, the mean level of Lp(a) was 25.5 ± 6.4 in men and women combined (25.9 ± 8.4 in men and 24.5 ± 9.2 mg/dl in women) compared with 12.3 ± 1.4 mg/dl ($p < 0.05$) in those with no medical conditions.

Subjects were categorized according to the number of medical conditions they reported, and mean Lp(a) values were calculated for each group (Fig. 1). Tests were carried out to determine whether there was a linear trend in Lp(a) levels across categories with the use of regression analyses and with age in men, women, and the combined sample adjusted for (Fig. 1). A significant trend for increasing Lp(a) levels was noted as the number of conditions increased in the entire group ($p = 0.03$). In women alone, this relationship was also statistically significant ($p = 0.04$). In men, however, this relationship was only of borderline significance ($p = 0.10$).

Similar analyses were carried out for the measurements of apo A-I, apo B, cholesterol, triglycerides, HDL

cholesterol, LDL cholesterol, cholesterol/HDL cholesterol, and apo A-I/apo B ratios. No statistically significant trend was found for any of these measurements (data not shown). Only total triglycerides showed a marginal increasing trend with number of medical conditions for men ($p = 0.07$). The results for women were not significant ($p = 0.48$).

Study 2. In the 69 women before treatment, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides averaged 226.4 ± 5.0, 62.2 ± 1.6, 150.8 ± 5.0, and 96.7 ± 4.8 mg/dl, respectively. Concentrations of serum HDL_2 cholesterol, apo A-I, apo B, and very-low-density lipoprotein (VLDL) were 15.2 ± 0.8, 169.1 ± 4.3, 83.9 ± 3.1, and 13.9 ± 1.1 mg/dl, respectively. Serum Lp(a) averaged 15.2 ± 1.8 mg/dl, but Lp(a) measurements varied significantly in the different groups at baseline. At baseline, Lp(a) did not correlate with any of the above parameters but showed a borderline negative correlation with HDL cholesterol ($r = -0.21$, $p = 0.08$).

The effect of various treatments for 6 months are depicted in Fig. 2. Exercise alone had a minimal and nonsignificant effect on Lp(a) (decrease of 2.5%). With estrogen, there was a 31.7% and a 26.9% decrease, respectively, for conjugated equine estrogen alone and for conjugated equine estrogen plus exercise and a 10.6% decrese associated with the use of transdermal estrogen alone. None of these differences was significant. Using repeated-measures analysis of variance and combining the two groups with conjugated equine estrogen together, the decrease in Lp(a) with treatment was of borderline significance ($p = 0.08$). The decrements in Lp(a), however, were not in those patients who had higher baseline values of Lp(a). In women who had Lp(a) values >10 mg/dl, Lp(a) decreased by only 8%, whereas in those women with baseline levels <10 mg/dl Lp(a) decreased by 37.8%.

Analysis of the changes in the other lipids and lipoproteins with treatment showed that exercise alone and transdermal estrogen did not affect any of the parameters (data not shown). After conjugated equine estrogen, in the groups without and with exercise, HDL cholesterol increased significantly by 14.9% and 13.9%, respectively (62.8 ± 2.8 to 71.8 ± 2.9 and 60.8 ± 2.4 to 69.1 ± 3.2 mg/dl, $p = 0.02$). The observed decreases in LDL cholesterol in these groups (13.1% and 11.1%) were not significant ($p = 0.18$) because of wide individual changes. However, triglycerides increased significantly by 40.6% and 48% from 92.6 ± 8.7 to 130.4 ± 12.2 mg/dl and 102.0 ± 9.8 to 153.3 ± 14.5 mg/dl, respectively ($p = 0.001$). VLDL concentrations also increased significantly by 49% and 51.7%, from 12.1 ± 1.6 to 19.3 ± 3.2 and 14.9 ± 3.1 to 20.6 ± 2.3 mg/dl, respectively ($p = 0.004$).

The observed, marginally significant decreases in Lp(a) levels with conjugated equine estrogen ($p = 0.08$) did not correlate with changes in HDL ($r = 0.02$) or LDL cholesterol ($r = 0.15$). However, after conjugated equine estrogen the changes in Lp(a) showed significant negative correlations with the increases in triglycerides ($r = -0.49$, $p = 0.001$) and VLDL ($r = -0.31$, $p = 0.06$).

Comment

Although Lp(a) was identified as a specific lipoprotein more than 20 years ago, little attention has been given to its clinical importance until recently. At present, its measurement is of considerable interest because of its apparent independent predictive value for cardiovascular disease risk. It has been reported that when Lp(a) levels in men are >30 mg/dl, the risk of myocardial infarction is increased twofold, and if Lp(a) and LDL are both increased, as compared with men with normal levels, the risk increases fivefold.[21] However, no study to our knowledge has specifically focused on this measurement in women. Although it has been known for some time that Lp(a) levels have a skewed distribution in white subjects and that levels are largely determined by genetic factors, we wished to examine closely the effect of sex, age, exercise, and estrogen on levels of Lp(a), focusing specifically on women.

Baseline levels of Lp(a) in women did not correlate well or consistently with any other lipids or lipoproteins we measured. In our first study Lp(a) correlated with triglycerides, and in the second group of younger women HDL cholesterol correlated in a negative direction with Lp(a). In the second study, with a statistical model created to explain the variation in levels of Lp(a) at baseline and based on other lipid and lipoprotein measurements, HDL cholesterol, followed by total triglycerides and cholesterol together could only account for 14.1% of the variability in Lp(a) levels. In these studies we showed that neither sex or age influenced levels of Lp(a).

Levels of Lp(a) were higher in men and women with stroke and transient ischemic attacks and were slightly higher when cardiac disorders were present. However, as the number of cardiovascular disease conditions increased, we observed a statistically significant trend of increasing Lp(a) levels. This effect was more striking for women than for men. Therefore these data are consistent with the notion that Lp(a) has a predictive value for cardiovascular disease in women. Because Lp(a) levels are known not to be affected by diet or the reduction in cholesterol with drug ingestion,[22-28] we suggest that measurements of Lp(a) may prove to be a valuable stable marker for the risk of cardiovascular disease.

Of considerable interest was the suggestion that levels of Lp(a) in patients with stroke and transient ischemic

attacks were of even greater predictive value than for cardiac disorders. Previous reports have linked measurements of Lp(a) with thrombotic stroke.[24] This is consistent with the physiologic feature of Lp(a) in that apo(a) and plasminogen have homology; elevated levels of Lp(a), by mimicking the role of plasminogen, may interfere with fibrinolysis,[25] thus promoting a particular propensity for thrombosis.

In this study we also wished to determine if life-style changes in women, such as exercise, would affect Lp(a) or if estrogen replacement could lower levels of Lp(a). This knowledge would be of importance for two reasons. First, we felt it would be important to determine if exercise or estrogen would potentially alter the predictive value of Lp(a). Cholesterol and triglyceride measurements, for example, are affected by both exercise and estrogen. Second, we wished to determine if these factors would lower Lp(a) such that, by so doing, a woman could potentially lower her risk of cardiovascular disease.

We found that exercise alone had no effect. Oral estrogen in the form of conjugated equine estrogen decreased Lp(a) levels somewhat ($p = 0.08$), but there was great variation in levels, which is in keeping with the known skewed distribution of Lp(a). Although the changes in the other lipids and lipoproteins were generally in the directions expected with the use of oral estrogen,[26] not all parameters achieved statistical significance, in part because of individual variation. However, the data suggest that oral estrogen may not be able to reduce the risk associated with high levels of Lp(a) because patients with higher values actually had the smallest changes in Lp(a) and the largest reductions in Lp(a) were seen in patients with low Lp(a) values.

The decrease in Lp(a) levels with conjugated equine estrogen use was significantly correlated with increases in triglycerides and VLDL. This suggests that oral, but not transdermal, estrogen may interefere somewhat with the synthesis of Lp(a) through a mechanism involved with synthesis of VLDL and triglycerides but not HDL. Lp(a) is known to be synthesized in the liver, and indeed messenger ribonucleic acid for Lp(a) has been detected in hepatic cell lines.[11, 12] These data support the concept that oral estrogen may have a minor influence on hepatic synthesis of Lp(a). Further elucidation of the mechanisms of this relationship may be important for understanding the physiologic metabolism of Lp(a).

In summary, Lp(a) levels appear to have no strong relationship to age, sex, or exercise and are primarily influenced by genetic factors involved in the synthesis of apo(a). By phenotyping apo(a) and identifying specific alleles, even more information may be forthcoming in the future. For example, it has been determined[10] that Lp^{S2} is associated with higher Lp(a) levels and higher cardiovascular disease risk as compared with Lp^{S4}, which is associated with low Lp(a) levels.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Leon Speroff, Portland, Oregon (Fellow). Studies in the literature indicate that elevated levels of Lp(a) lipoprotein are associated with atherosclerotic heart disease in both men and women. What is intriguing is that this association is independent of the cholesterol-lipoprotein profile (although there is a weak correlation with triglycerides) and the familiar risk factors such as age and diet.[1] Lp(a) may be involved in both atherogenesis and thrombogenesis. The atherogenetic mechanism is similar to that of LDL: oxidative modification and uptake by the macrophage scavenger cell receptors in the intima of blood vessels. Thrombogenesis may be the result of the homology of Lp(a) with plasminogen, resulting in impaired fibrinolysis.

The purpose of this study was twofold:(1) to gather cross-sectional data seeking to confirm the lack of association between Lp(a) and cardiovascular disease risk factors and (2) to assess in women the effects of estrogen therapy and interventions in exercise habits.

Lp(a) was measured by an enzyme-linked immunosorbent assay technique and a purified polyclonal antibody. In the cross-sectional part of the investigation a random blood sample was assayed, and in the intervention part Lp(a) was measured before and after exercise or estrogen treatment. The results confirm findings in the literature of a lack of association between Lp(a) levels and sex and age and higher Lp(a) levels in men and women with cardiovascular disease factors. Exercise alone had no influence on Lp(a) levels, whereas oral and transdermal estrogen lowered Lp(a) levels, although the changes did not reach statistical significance. The authors concluded that the data are consistent with Lp(a) having predictive value for cardiovascular disease, and because estrogen had the smallest impact in women with the highest Lp(a) values, estrogen may not be able to affect the risk associated with Lp(a).

Lp(a) is a combination of LDL and two protein moieties, apo(a) and apolipoprotein B-100, which are covalently linked together. Apo(a) exists in different isoforms, varying in size and number. Lp(a) is also a glycoprotein, and because of its high carbohydrate content, it is soluble in aqueous media.[2] Like other glycoproteins, the carbohydrate component can vary. Therefore this structure has tremendous variability, and it is not surprising that measurements of this substance with one antibody in an immunoassay reveal great variability, up to 1000-fold between individuals, although levels remain relatively constant in the same individual. To compensate for this variability, studies must include large numbers of subjects. This probably explains the failure of the cross-sectional investigation to find significant elevations in men with cardiovascular disease factors. This variability between individuals is also important when an intervention study with before-and-after measurements is designed. Because levels are relatively constant within individuals, subjects should serve as their own controls. Group analysis, as performed in this study, would again require large numbers of subjects. In the intervention part of this study, the baseline Lp(a) levels differed greatly between groups, and the effect of estrogen did not reach statistical significance within each group. Perhaps larger numbers would have revealed some impact of exercise and, more likely, a greater effect of estrogen.

The description of methods did not specify with full accuracy how the subjects were chosen. Factors that affect cardiovascular disease need to be controlled to detect differences in one variable. Beside age and drugs, these factors include diet, level of fitness and body composition, past estrogen use, and the baseline lipid state. It is noteworthy that HDL levels were relatively high in the second part of the study, indicating a rather (cardiovascularly) healthy group of women. Estrogen treatment appears to have a greater impact on cardiovascular disease protection in women with risk factors and unfavorable lipoprotein profiles. An analysis of estrogen effect on Lp(a) should include sufficient numbers of women to stratify the impact according to risk factors and the lipoprotein profile. Because Lp(a) levels appear to be primarily genetically determined (i.e., not affected by diet and drugs), it can be argued that Lp(a) is a more stable marker and not influenced by the usual cardiovascular risk factors. Nevertheless, this must be documented.

The actual levels of Lp(a) measured are a little concerning. In general the literature indicates that relatively high levels of Lp(a) must be present to indicate an association with cardiovascular disease, and only 10% to 15% of the white population carry pathologic levels of Lp(a).[3-6] In one study levels had to be >365 mg/dl to have twice the rate of coronary heart disease.[7] The mean levels in this study range from 11.2 to 53 mg/dl. The group means in the second part of the study were relatively low. Again these differences probably reflect the great variability in Lp(a), and they underscore the need for large numbers of subjects. Although this study indicated the greatest impact of estrogen in women with lower Lp(a) levels, it is unlikely that this study included any women with very high

levels, levels with the strongest correlation wtih cardiovascular disease.

In spite of the difficulties, this is an important contribution. It has been suggested that Lp(a) ranks with the HDL/total cholesterol ratio as the best predictor of coronary artery disease.[8] This study reinforces the observation that genetically determined plasma levels of Lp(a) have predictive value for cardiovascular disease, and this is, to my knowledge, the first investigation of the impact of estrogen.

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Dr. Norman Gant, Dallas, Texas. One of the questions that came to mind concerns the very low values for Lp(a) that you observed in the older patients. Were they not in fact values obtained in the survivors? Had they not already preselected themselves? Those with higher levels possibly had already died before getting to "vacation village."

I was just wondering if you've had an opportunity to look at younger, middle-aged men who might be more at risk. In other words, have you looked at some younger people who haven't yet canceled themselves out of the study?

Dr. Howard L. Judd, Los Angeles, California. We've just finished a multicenter study in which we looked at lipids with various estrogens. The responses of the lipids to hormonal manipulation were influenced by the baseline level. If the HDL was low, then the response was greater than when the HDL was high, and the opposite was true for LDL. Did you have an opportunity to look at baseline levels and check responses of your lipids in regard to manipulations that you observed?

Dr. Frederick Naftolin, New Haven, Connecticut. The evidence that exercise can have a measurable impact on the outcome of a woman's life at the point we are examining is decreasingly small, if at all visible. I think that we shouldn't lose this opportunity to see that here again is evidence that genetic constitution and hormonal levels are the primary motivators of such things as cardiovascular risk. We shouldn't lose the opportunity to see the futility of advising elderly women to undertake exercise regimens to determine the outcomes of their cardiovascular lives.

Dr. James M. Roberts, San Francisco, California. I have to object to one of your statements—unless I misread the data. You stated that there was a close correlation between triglycerides and Lp(a) levels. The r values I saw was 0.22; thus R^2 is 0.04, which means that only 4% of variation could be accounted for by the relationship, which doesn't seem to me to be a high correlation.

Dr. Lobo (Closing). To answer Dr. Speroff's first question, the acknowledged variability in the levels of Lp(a) is not really an assay problem. It is really a genetic trait that determines the levels of Lp(a) because of various phenotypes of apo(a).

I would have preferred to incorporate more patients into both studies. Nevertheless, in the literature those studies that have reported the different ranges of odds ratios for the association of Lp(a) levels with cardiovascular disease have had numbers of patients in the same range that we have reported here.

The difficulty in these types of studies is that there is greater variability in Lp(a) levels because of differences in the genetic background. A skewed distribution of values occurs in white subjects. However, this does not occur in black persons who, for example, have a nice bell-shaped distribution. So my contention is that it is actually quite difficult to carry out studies on Lp(a) because of its variability.

However, one should note that in spite of this we saw a positive trend in the Leisure World cohort, between an increasing number of medical conditions and higher levels of Lp(a). This was significant in both men and in women. We didn't see these trends, however, for what are known to be established risk factors such as apo A-I, LDL, and HDL. In this model you could almost argue that Lp(a) values were actually better than the established risk factors.

I think Dr. Gant is absolutely right. It was our conclusion that the individuals at greatest risk had died. We were studying a healthier survivor population where we still see some semblance of Lp(a) constituting a risk, but our study does not address the risk of the man who is 45 years old and has had three myocardial infarctions. Because of the variation in Lp(a) levels, I think the future in this field is really to specifically identify apo(a) phenotypes. For example, by measuring the S-2 phenotype as opposed to the S-4 phenotype where the levels are higher, one could pick up about 14% of the white population who have this trait. Those would be the people to identify who are at risk for thrombosis and atherogenesis related to lipoproteins.

Dr. Speroff asked me about selection of the variables and selection of patients. In the cross-sectional study the subjects were closely matched, particularly by age,

and had no other confounding diseases such as diabetes mellitus or hypertension. The baseline lipoprotein measurements are typical of what has been reported for men and women.

In our prospective study the younger individuals were specifically screened for not having risk factors because we wished to assess life-style variables. So this was a very fit, young, healthy population. Diet was controlled and no estrogen was being used. The high HDL levels at baseline really confirm the subjects' cardiovascular health. We therefore wished to study if exercise or estrogen would alter Lp(a) levels in this healthy population.

Indeed, our conclusion here is that within an individual Lp(a) is a very stable marker. Estrogen probably does influence Lp(a) levels but only by a minor degree. Exercise does not appear to have an effect. So it really reflects one's inherent propensity for cardiovascular risk.

Again, I thank Dr. Gant for his very intuitive insight. I agree with him. We were dealing largely with a survival population. Dr. Judd had a question related to baseline variability. In our manuscript we have actually included these data. Whereas the influence of baseline values is probably true for changes in HDL and LDL, this was not the case for changes in Lp(a). We looked at those patients with higher values and lower values and saw the same magnitude of borderline change in the two groups for Lp(a).

Dr. Naftolin, I think the question on exercise is still open to debate. I understand there is controversy in this area, but again we felt that in this model Lp(a) was not influenced by exercise.

For Roberts' comments challenging the concept of VLDL, triglyceride, and Lp(a) changes, indeed these are matching correlative analyses, and the statistics were not strong, although changes were significant because of the larger sample size. The point we're trying to make is that because the complementary deoxyribonucleic acid for apo(a) has been constructed from a hepatic library of ribonucleic acid, suggesting that apo(a) production is hepatic in origin, we feel that some common hepatic metabolism may be involved with these lipoproteins. I guess I could be challenged enough to say that these associations remain speculative.

Lipid and apolipoprotein abnormalities in hirsute women

I. The association with insulin resistance

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OBJECTIVE: We attempted to evaluate apolipoprotein lipid levels in hirsute hyperandrogenic women and to clarify the influence of insulin resistance versus endogenous sex steroids on these profiles.
STUDY DESIGN: Forty-seven women seen for evaluation of hirsutism in the Reproductive Endocrine Clinic were contrasted with 15 normal women. Thirty-one patients were treated with the gonadotropin-releasing hormone analog, leuprolide acetate, 7.5 mg intramuscularly monthly for 3 months. Insulin resistance was assessed before and after GnRH analog administration. Mean concentrations were compared with the Student *t* test and analysis of variance.
RESULTS: Hirsute women were heavier and had higher systolic and diastolic blood pressures, more menstrual irregularities (all $p < 0.01$), and higher waist/hip ratios ($p < 0.05$). They had higher triglyceride, very-low-density lipoprotein cholesterol, and apolipoprotein C-III levels and lower high-density lipoprotein cholesterol concentrations (all $p < 0.01$). They had lower apolipoprotein A-I/A-II ratios ($p < 0.01$). After administration of GnRH analog, androgen and estrogens were suppressed and insulin resistance was unaltered. Insulin correlated with apolipoprotein lipid abnormalities.
CONCLUSION: Lipid and apolipoprotein abnormalities in these hirsute women seem to be associated more with insulin resistance than with endogenous androgens or estrogens. (AM J OBSTET GYNECOL 1992;166:1191-7.)

Key words: Insulin resistance, gonadotropin-releasing hormone analog, hirsutism, apolipoprotein

Hirsutism and acne are clearly more than cosmetic concerns.[1] These physical features, expressions of androgen excess,[2,3] signal metabolic aberration.[1,4] Women with polycystic ovary syndrome, the most common endocrine disorder associated with hirsutism, have insulin resistance whether or not they are obese.[4,5] They frequently have male pattern obesity,[6,7] higher levels of free testosterone than normal women,[1,7] and less favorable lipoprotein lipid levels in plasma.[8] All of these are risk factors for coronary vascular disease in women.[9,10]

To investigate one of the cardiovascular risk factors present in women with hirsutism, we began studying their lipid metabolism.[1] We have found that they have higher concentrations of triglycerides and lower concentrations of high-density lipoprotein (HDL) cholesterol than normal women, even when matched for body weight.[1,8] By cross-sectional analysis in a separate population, these altered levels of lipoprotein lipids have been found to be associated independently with both androgen and insulin excess.[7] In an attempt to differentiate between these two possible effects, a group of hirsute women was tested with gonadotropin-releasing hormone (GnRH) analog to remove the possible effect of endogenous gonadal hormones. Results of this intervention study show that insulin or insulin resistance exerts a greater effect than gonadal hormones on the levels of lipids and apolipoproteins in plasma of hyperandrogenic women.

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Subjects and methods

Patients. Forty-seven women complaining of hirsutism were evaluated by history, physical examination, and determination of sex steroids, fasting lipoprotein lipids, apolipoproteins, and parameters of insulin resistance. They were compared with a control group of 15 nonhirsute, regularly menstruating women sampled in the proliferative phase of the menstrual cycle. Patients with Cushing's syndrome, hypothyroidism, androgen-secreting tumors, or prolactinomas were excluded. All women had documented hyperandrogenism, defined as an increased level of one or more of the following: free and albumin-bound testosterone,

Table I. Clinical characteristics

	Hirsute (n = 47)	*Normal (n = 15)*
Age (yr)	31.5 ± 6.6	32.1 ± 8.9
Weight (lb)	204.6 ± 54.7*	129.5 ± 13.8
Height (in)	65.3 ± 2.5	65.0 ± 2.1
Systolic blood pressure (mm Hg)	121.6 ± 11.0†	109.3 ± 14.5
Diastolic blood pressure (mm Hg)	76.7 ± 7.5‡	66.7 ± 8.0
Waist (cm)	94.7 ± 17.0†	68.9 ± 5.8
Hips (cm)	117.3 ± 18.2	94.0 ± 4.4
Waist/hip ratio	0.81 ± 0.07§	0.73 ± 0.04
Hirsutism	24.9 ± 7.8*	2.7 ± 5.2
Menses (No./yr)	8.0 ± 3.0†	12.0 ± 0
Diet score‖	29.5 ± 40.0	20.0 ± 36.3

*$p < 0.0001$.
†$p < 0.01$.
‡$p < 0.001$.
§$p < 0.05$.
‖Score based on immediate recall—index of fat and fiber content.

Table II. Serum glyocprotein, androgen, and estrogen levels

	Hirsute (n = 47)	*Normal (n = 15)*
Luteinizing hormone (mIU/ml)	13.3 ± 15.9	8.2 ± 5.3
Follicle-stimulating hormone (mIU/ml)	9.4 ± 3.9	8.0 ± 3.1
Testosterone (ng/dl)	90.7 ± 38.9*	29.6 ± 10.8
Unbound testosterone (ng/dl)	22.3 ± 20.7†	4.6 ± 3.4
Androstenedione (ng/dl)	3.4 ± 2.3‡	1.8 ± 0.3
Sex hormone–binding globulin (nmol/L)	44.3 ± 24.8‡	108.5 ± 39.1
DHEAS (μg/ml)	2.6 ± 2.0*	1.0 ± 0.5
Estradiol (pg/ml)	58.5 ± 40.3†	103.4 ± 67.6

Values are mean ± SD.
*$p < 0.0001$.
†$p < 0.05$.
‡$p < 0.001$.

dehydroepiandrosterone sulfate (DHEAS), or androstenedione. All women were in excellent general health. For 3 months before the study none had taken any medications known to affect pituitary-gonadal function or carbohydrate or lipid metabolism. Smokers were excluded. Women with blood pressure >140 mm Hg systolic or >90 mm Hg diastolic were excluded. None consumed excessive amounts of alcohol, and none were vegetarians or eating any other special diet. Diet history, exercise frequency, and family history of cardiovascular disease were recorded and scored for each patient as described previously.[1] Hirsutism was graded as reported by Ferriman and Gallwey. Acne was graded as recorded by Pleiwig and Kligman.[1] Obesity was defined as >120% ideal body weight according to current Metropolitan Life Insurance Company tables. If the 17α-hydroxyprogesterone concentration was >2.0 mg/ml, a corticotropin stimulation test with 25 μm corticotropin was performed. 17α-Hydroxyprogesterone was measured at baseline and at 60 minutes to rule out 21-hydroxylase deficiency.

Table III. Lipids, lipoprotein cholesterol, apolipoproteins, and ratios

	Hirsute (n = 47)	*Normal (n = 15)*
Triglycerides	115.3 ± 79.0*	57.6 ± 28.8
Cholesterol	193.1 ± 36.2	173.4 ± 25.5
HDL cholesterol	47.5 ± 8.3†	54.8 ± 17.2
LDL cholesterol	121.9 ± 32.8	104.8 ± 28.1
VLDL cholesterol	23.0 ± 15.8*	12.7 ± 7.5
Apo A-I	140.0 ± 15.4	151.3 ± 30.4
Apo A-II	78.2 ± 12.4	72.1 ± 10.9
Apo B	96.7 ± 29.7	84.2 ± 29.6
Apo C-III	11.6 ± 4.7†	9.6 ± 2.3
Apo E	15.4 ± 4.7	13.7 ± 3.8
Apo C III-HS	6.1 ± 1.8	5.8 ± 2.2
Apo C III-HP	4.5 ± 3.6‡	3.0 ± 1.5
Apo A I/apo B	1.8 ± 1.8	1.9 ± 0.6
Apo A-I/apo C-III	13.8 ± 5.2	16.7 ± 3.8
Apo A-I/apo A-II	1.8 ± 0.3†	2.1 ± 0.4
Apo C-III/apo E	0.77 ± 0.25	0.77 ± 0.24
LDL cholesterol/apo B	1.4 ± 0.94	1.2 ± 0.2
HDL cholesterol/apo A-I	0.34 ± 0.05	0.38 ± 0.08
LDL cholesterol/HDL cholesterol	2.7 ± 0.9‡	1.9 ± 1.0
Choelsterol/HDL cholesterol	4.2 ± 1.2‡	3.2 ± 1.1

Values are in milligrams per deciliter, mean ± SD. *apo C-III-HS*, apo C-III in heparin-Mn†† supernate; *apo C-III-HP*, apo C-III in heparin-Mn†† precipitate.
*$p < 0.001$.
†$p < 0.01$.
‡$p < 0.05$.

Thirty-one hirsute women were given GnRH analog (Lupron Depot, 7.5 mg intramuscularly, Tapp Pharmaceutical, Chicago) and were reevaluated in a fashion identical to baseline after 3 monthly injections of GnRH analog. This study was approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center.

Insulin resistance. Insulin resistance was determined by an intravenous bolus of 0.1 U/kg of regular insulin and determination of glucose disposal.[11]

Sex steroids and glycoprotein hormones. Serum follicle-stimulating hormone, luteinizing hormone, testosterone, unbound testosterone, sex hormone–binding globulin, DHEAS, androstenedione, and estradiol were analyzed by radioimmunoassay by well-established, previously reported methods.[7] When serum estradiol was below the low sensitivity of the assay (20 pg/ml), it was assigned a value of 20 for statistical analysis.

Glucose, insulin, C-peptide. Glucose was measured

Table IV. Serum androgen and estrogen levels at baseline and after 3 months of GnRh analog

	Baseline (n = 31)	*After 3 mo of GnRH analog (n = 31)*
Testosterone (ng/dl)	98.5 ± 43.8	55.2 ± 20.7*
Unbound testosterone (ng/dl)	34.2 ± 21.7	17.1 ± 10.2*
Sex hormone–binding globulin (nmol/L)	45.2 ± 26.3	36.9 ± 22.0
DHEAS (μg/ml)	2.2 ± 1.1	2.1 ± 1.2
Androstenedione (ng/dl)	3.28 ± 1.4	1.9 ± 0.8*
Estradiol (pg/ml)	63.2 ± 44.9	26.1 ± 11.4†‡

*$p < 0.01$.
†$p < 0.05$.
‡Sensitivity 20 pg/ml.

Table V. Lipids, lipoprotein cholesterol, apolipoproteins, and ratios

	Baseline (n = 31)	*3 months GnRH analog (n = 31)*
Triglycerides	123.2 ± 92.1	115.1 ± 75.7
Cholesterol	197.7 ± 37.1	199.4 ± 43.7
HDL cholesterol	48.6 ± 7.8	52.3 ± 20.6
Apo A-I	141.3 ± 15.9	142.1 ± 32.4
Apo C-III	12.0 ± 5.1	11.7 ± 4.2
Apo C-III-heparin precipitated	6.2 ± 1.7	5.7 ± 1.3
Apo A-I/apo A-II	1.8 ± 0.2	1.7 ± 0.5
LDL cholesterol/HDL cholesterol	2.6 ± 0.9	2.7 ± 1.0
Cholesterol/HDL cholesterol	4.2 ± 1.1	4.2 ± 1.4

Values are in milligrams per deciliter, mean ± SD. p Value not significant.

Table VI. Glucose, insulin, and C-peptide values at baseline and after insulin bolus before and after GnRH analog

	Baseline (n = 31)	*3 months GnRH analog (n = 31)*
Fasting		
Glucose (mg/dl)	83.9 ± 12.3	83.8 ± 10.1
Insulin (uU/ml)	24.6 ± 28.8	21.2 ± 12.6
C-peptide (ng/ml)	2.2 ± 3.2	2.0 ± 1.9
Nadir		
Glucose	40.7 ± 14.1	39.2 ± 14.8
Glucose time (min)	15.2 ± 6.7	13.5 ± 6.8
Insulin	20.2 ± 18.4	18.9 ± 11.2
Insulin time (min)	−7.0 ± 10.4	−10.2 ± 5.7
C-peptide	0.89 ± 1.7	0.79 ± 0.5
C-peptide time (min)	24.3 ± 7.3	22.4 ± 8.1
Dissociation constant	0.0178 ± 0.00978	0.01957 ± 0.00948

p Value, not significant.

with a Beckman glucose analyzer II (Brea, Calif.). Insulin and C-peptide was measured by radioimmunoassay, again by previously reported methods.[7] Interassay variation was ≤9%.

Lipids, lipoprotein cholesterol, and apolipoproteins. Plasma total cholesterol (CHOP/PAP, Boehringer, Mannheim, Germany) and triglyceride (kits for total triglyceride and free glycerol, Abbott Laboratories, Diagnostics Division, Irving, Tex.) concentrations were determined enzymatically on an Abbott VP-Super System analyzer. Both assays were standardized with serum calibrators and control samples supplied by the Centers for Disease Control, Atlanta. Cholesterol contents of very-low-density (VLDL), low-density (LDL), and HDL lipoproteins were measured according to the procedures of the Lipid Research Clinics Program. Apolipoprotein analyses were carried out by electroimmunoassays as previously described[12] for apo A-I and apo A-II, apo B, apo C-III, and apo E. Apo C-III was measured in whole serum and in heparin-precipitated and heparin supernate lipoproteins. The heparin-precipitated (VLDL and LDL) and nonprecipitated (HDL) lipoproteins were isolated by a modification of the heparin-Mn^{++} method in that a Mn^{++} concentration of 0.092 mol/L was used instead of 0.046 as originally described. The apo C-III heparin supernate/heparin-precipitated apo C-III ratio represents a useful criterion for assessing the efficiency of triglyceride-rich lipoprotein degradation.

Statistics. Mean concentrations were compared by the Student t test and analysis of variance. Triglyceride and fasting insulin value were logarithmically normalized before analysis. Pearson product moment correlation coefficients were computed. All computations were made with SAS (Statistical Analysis Systems, Inc., Cary, N.C.). Significance was set at $p < 0.05$.

Results

Clinical parameters of the hirsute women contrasted with controls are shown in Table I, and their average hormone concentrations are contrasted in Table II. As expected, we found that the hirsute women were heavier and had higher systolic and diastolic blood pressures, higher waist/hip ratios, and more menstrual irregularity. We documented higher concentrations of androgens (androstenedione, testosterone, unbound testosterone, and DHEAS). We found significantly higher concentrations of plasma triglycerides, VLDL

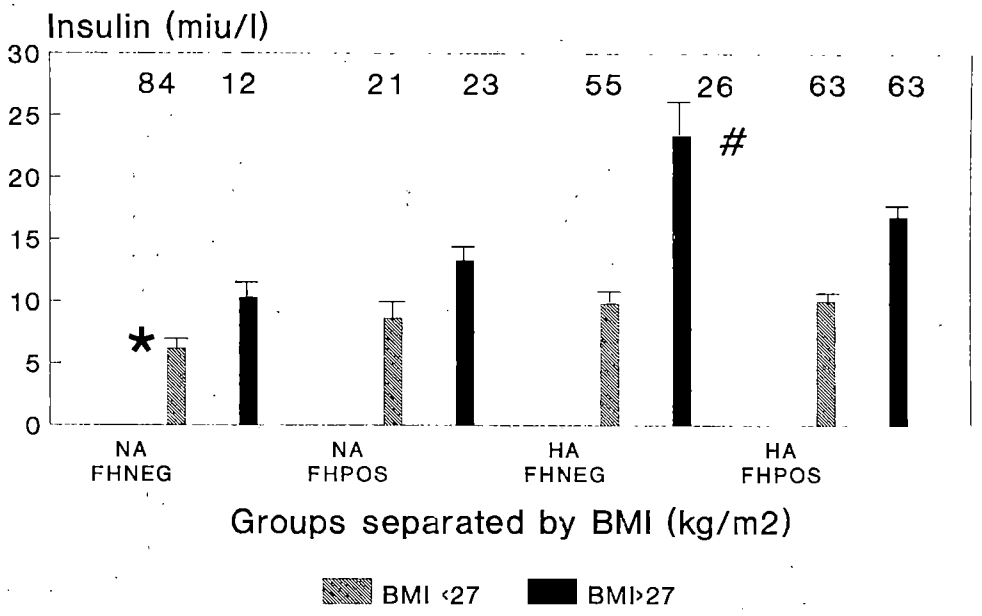


Fig. 1. Fasting insulin concentrations in 204 hyperandrogenic *(HA)* and 140 case-control women *(NA)*, stratified by body mass index *(BMI)* and family history of diabetes *(FHNEG, FHPOS)*. *Number symbol*, $p < 0.01$, against other groups; *asterisk*, $p < 0.01$, against all other groups.

Table VII. Correlation of logarithmically normalized fasting insulin and unbound testosterone with lipoprotein lipids, apolipoproteins, and glucose before and during GnRH analog in hirsute women

	Logarithmically normalized fasting insulin		*Unbound testosterone**	
	Baseline	*GnRH analog at 3 mo (p value)*	*Baseline*	*GnRH analog at 3 mo (p value)*
Fasting glucose	0.50 (0.004)	0.42 (0.02)	0.30	0.19
Logarithmically normalized triglycerides	0.34 (0.06)	0.59 (0.0001)	0.22	0.31
VLDL cholesterol	0.22 (NS)	0.51 (0.006)	0.07	0.13
Apo C-III	0.22 (NS)	0.38 (0.05)	0.07	0.08
Apo E	0.16 (NS)	0.40 (0.04)	−0.09	0.32
Apo C-III heparin precipitated	0.14 (NS)	0.48 (0.01)	0.12	0.07
Apo C-III supernatant	−0.44 (0.02)	−0.41 (0.04)	−0.25	−0.36

NS, Not significant.
**p* Value not significant.

cholesterol, and apo C-III and significantly lower levels of HDL cholesterol in the hirsute women compared with the normal women (Table III). They were also found to have lower levels of apo A-I and higher levels of apo A-II with a resulting significantly lower apo A-I/apo A-II ratio than control subjects (Table III).

After 3 months of GnRH analog treatment, there was a significant suppression of androgen and estrogen levels (Table IV), but there were no significant changes in average lipid, lipoprotein cholesterol, and apolipoprotein concentrations (Table V). In contrast to significant changes in the plasma levels of gonadal hormones, the insulin resistance remained unaltered after 3 months of GnRH analog treatment in the hirsute women (Table VI). Average body weight was unchanged during treatment. Correlation coefficients between logarithmically normalized fasting insulin concentrations and altered lipoprotein and apolipoproteins are contrasted with correlations between unbound testosterone and the same lipid parameters both before and at the end of the third month of GnRH analog in Table VII. Correlation coefficients were stronger between logarithmically fasting insulin and triglycerides, VLDL cholesterol, apo C-III, apo B, and heparin-precipitated apo C-III after 3 months of treatment.

Comment

We have confirmed that hirsute hyperandrogenic women have lower HDL cholesterol and higher triglyceride and VLDL cholesterol concentrations than

normal women, and we have expanded our observations to demonstrate that they also have unfavorable apolipoprotein profiles. We have used an analog of GnRH as a powerful research and therapeutic tool to remove endogenous ovarian androgen and estrogen production to observe the subsequent effects of removal of gonadal hormones on lipoprotein and apolipoprotein metabolism. We observed, as has been reported previously,[4, 5] that insulin resistance accompanied by elevated fasting insulin levels and delayed glucose disposal is common when hirsutism is present. We found, as has been documented, no apparent effect of GnRH analog on either adrenal androgen production or insulin resistance after 3 months of suppression of the hypothalamic-pituitary-gonadal axis.[13-15]

The abnormalities in plasma levels of triglyceride-rich lipoprotein constituents and HDL cholesterol, previously shown to be correlated with androgens and insulin,[7] seem to be associated more with insulin resistance than with plasma levels of androgens and estrogens. Their lipid and apolipoprotein profiles demonstrate the same pattern as that seen in other states of insulin resistance with varying degress of glucose tolerance.[16] The fact that these profiles are unaltered during GnRH analog therapy, although parameters of insulin resistance were unchanged, suggests that insulin has a more powerful influence on lipid and apolipoprotein metabolism in hirsute women than does either endogenous gonadal androgen or estrogen.

In this study we noted that most women had good ovarian suppression by the analog (average estradiol concentration on GnRH analog was near castrate levels), but in spite of this the average testosterone concentrations remained at the upper limits of or slightly above normal. This is probably a reflection of testosterone's origins from adrenal production or peripheral conversion of its precursors. We cannot rule out an effect of either gonadal estrogen or androgen on lipid metabolism in these patients; however, we can conclude that if there is an effect of either of these hormones, the effects seem to be overshadowed by the effect of elevated insulin found as a consequence of concurrent insulin resistance. A careful inspection of Table VII reveals that the association of fasting insulin with the lipid parameter becomes stronger after the influence of gonadal hormones is removed.

Recent evidence favors a powerful influence of insulin on ovarian androgen production. Both androgen and insulin may be important contributors to the problems of hirsutism[2, 17] and possibly acne through its action on insulin-like growth factor-I receptors.[17] Insulin excess associated with insulin resistance is linked both to elevated blood pressure and to upper-body-segment obesity.[18] Both of these are well-known risk factors for cardiovascular disease.[10] Women with polycystic ovary syndrome, the most common endocrinopathy associated with hirsutism, are prone to each of these abnormalities.[7]

Because some triglyceride-rich lipoproteins may have significant atherogenic potential,[19, 20] hirsute hyperandrogenic women need to be evaluated for this and other cardiovascular disease risk factors. Further evaluation of androgenized women may shed light on mechanisms for altered cardiovascular risk and may improve our understanding of reasons for the earlier onset and greater incidence of cardiovascular disease in men compared with women.

We thank Johnelle May for her concern for our patients and Lisa Harris for her expert technical assistance.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Brian A. Little, Montreal, Quebec, Canada (Fellow). More and more, polycystic ovarian syndrome and hirsutism are becoming mere manifestations of more complex metabolic diseases. Dr. Wild and his colleagues have added one more small piece to the puzzle. By the suppression of gonadotropin secretion through the administration of a GnRH agonist (leuprolide acetate [D-Leu^6, Pro^9-Het^{10}]), they have demonstrated that both lipid abnormalities and insulin resistance persisted in the face of reduced androgens. Although testosterone and androstenedione levels were reduced, they remained in the normal range. The DHEAS concentration was unchanged, remaining high.

Although there are some small niggling questions I have, most of my remarks will be to add support to the data and ask for comments on my interpretation of the data as they fit into the overall complex metabolic process.

First the questions: I noticed the clinical characteristics of the hirsute group included a weight differential of 75 pounds, the hirsute group averaging 204 pounds (normal 129) with a significantly greater waist/hip ratio (0.81 to 0.73). In spite of the fact that you have classified obese patients as those with >120% ideal body weight, how much did this obesity contribute to the results? If, for example, they are included in the hirsute group, it is well known that many of them have primary or marginal metabolic disease, e.g., abnormal carbohydrate metabolism or family history of diabetes and lipid abnormalities. For this reason it would be helpful to know what proportion of the group studied were obese or had a family history of diabetes Did you do any carbohydrate metabolism tests on your normal patients?

The heterogeneity of the study group is also manifest by the failure of the GnRH agonist to reduce the androgens (testosterone and androstenedione) below the normal level. This could be a function not only of the patient selection but also of the GnRH agonist used (leuprolide). For example, it could be that pharmacologic variants act somewhat differently. Nafarelin [D-Nal(2)^6], which is more potent, albeit when given in small doses (200 μg) intranasally to patients with polycystic ovary syndrome twice daily for 6 months, suppressed androgen levels below normal.[1] (This is all in the interest of explaining some of the problems that make this so complex.) What is Dr. Wild's view of the effect of different GnRH agonists and do they act differently on different tissues (e.g., pituitary, ovary, or adrenal)?

Fig. 1 shows another part of this intricate relationship. This is the comparison of fasting insulin concentrations in 204 hyperandrogenic women and 140 case controls, stratified by body mass index and family history of diabetes. In Fig. 1 fasting insulin is on the axis and the ordinate shows women grouped by body mass index of <27 kg/m^2 or >27 kg/m^2 with a negative or positive family history of non-insulin-dependent diabetes mellitus (confirmed by interview with the family) in nonandrogenic (case control) or hyperandrogenic patients (defined by a hirsutism score >16 or a testosterone value >1 SD above a group of normal fertile women). It can be seen that overall hyperandrogenic women had higher fasting insulin levels than did the control group, and family history–positive control women had higher values than their family history–negative counterparts. In women of normal weight (body mass index <27 kg/m^2) family history of diabetes mellitus made no difference in that the family history–positive control group was the same as the family history–positive and family history–negative hyperandrogenic group, except that only the family history–negative control group had lower fasting insulin levels than all others. Finally, obese women (body mass index >27 kg/m^2) who were family history negative had higher fasting insulin levels than any other group including both family history–positive hyperandrogenic and control women and family history–negative control subjects. In addition, Morris et al.[2] have gone on to show that abnormalities in serum lipoprotein concentrations in hyperandrogenic women are a reflection of the metabolic derangements associated with obesity.

If one could broadly equate fasting insulin with insulin resistance (and in this group the sum of insulin concentrations during an oral glucose tolerance reflected the findings for fasting insulin and equally relate such resistance to lipid abnormalities, I wonder if one would expect an effect of GnRH analog?

In any event, I congratulate Dr. Wild on making the most of a difficult result and would suggest to you all that at last we have a reason to take a family history. For I find that in patients with problems of abnormal cycling, infertility, and polycystic ovary syndrome, I have great success with the questions, "Are there sugar problems in the family? . . . Is there any heart disease? . . . What about hypertension?" The relationship between the first two have been addressed thoughtfully today. I predict the next abnormality to be related in this way may be hypertension, related in some way to the adrenal gland and kidney. As to what to do for such

genetic abnormalities, perhaps we shall hear more in the next meetings.

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Dr. Wild (Closing). Dr. Little, you addressed a number of areas, and I would like to share with you some of my personal observations and biases.

The first issue was an important one. What about the contribution of obesity to the entire process? There is no question of the independent effect of obesity on all the alterations that were seen. It's difficult to ferret out the independent effects of obesity, but what I would like to point out is that most studies that look at patients with polycystic ovary syndrome who are not obese find over and over again insulin resistance.

What's difficult to sort out when these evaluations are done is what is the role of diet interaction as well as the other potential confounder that you presented, the intriguing concept of family history as it relates to the entire process.

You brought up concerns—the bane of the existence of all of us that are interested in androgen disorders—the problem of heterogeneity. It's a constant problem, and I think it's important to realize that the insights we addressed here applied only to the population observed. There is no unanimity of definition of what the polycystic ovary syndrome really is, so we have to be careful to describe the exact population of inference. It is a heterogeneous disorder. Within the group seen, I'm sure there are significant endocrinopathies that can be discerned with further testing. I would like to point out that our study was an interim analysis. This was our first opportunity to arrive at power for inference. As numbers go up and as this protocol becomes modulated, maybe we can get better insight into those questions that are right now limited by power. The most important questions will be addressed by subgroup analysis; in our minds, in particular, the effect of adrenal androgen contribution versus ovarian androgen contribution.

You asked about the agonists and if they act differently. I don't have good data to relate to you, but it's my feeling that some of the reasons that these androgens came down close to normal but were not completely suppressed may be from the fact that there is a significant adrenal contribution that was not affected. This illustrates the beauty and specificity of the GnRH analog to begin to dissect out these questions. Another explanation is the issue of body weight and dose-response studies for the GnRH agonists. There is one article in the literature to my knowledge that looks at this with daily subcutaneous GnRH treatment, and it suggests that if dosages are adjusted for body weight, better suppression occurs. If the suppression is carried out >3 months, you may still get more suppression. We haven't been able to observe this effect as yet.

Your observations of family history data are very intriguing. It fits my bias that the interaction of dietary saturated fat with genetics may well be the prime mover in terms of aggravation of this phenomenon. You're absolutely right that interaction between insulin and blood pressure and the understanding of how they interact are exciting. Syndrome X is the pathophysiologic related condition, and as investigations continue, I think we can look forward to exciting inferences.

Elevated maternal plasma corticotropin-releasing hormone levels in pregnancies complicated by preterm labor

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OBJECTIVES: We investigated whether maternal plasma evels of the placental hormone corticotropin-releasing hormone are elevated in pregnancies complicated by preterm labor.
STUDY DESIGN: Mean maternal corticotropin-releasing hormone levels were studied in women who met specific criteria for preterm labor anc in women with normal pregnancies. Levels were also compared in the latent and active phases during term labor.
RESULTS: In pregnancies complicated by preterm labor, maternal corticotropin-releasing hormone levels were higher than in normal pregnancies; this elevation occurred before labor was diagnosed clinically ($p < 0.05$). When preterm labor was associated with infection, the mean levels were not elevated. Mean plasma levels were similar in latent and active phases during labor at term.
CONCLUSION: Maternal plasma corticotropin-releasing hormone levels are elevated in association with preterm labor. This elevation does not appear to be due to labor itself and may reflect an early activation of the placenta before the onset of preterm labor. (Am J Obstet Gynecol 1992;166:1198-207.)

Key words: Corticotropin-releasing hormone, preterm labor, placenta, fetus, hypothalamus, pituitary, adrenal

The hypothalamic hormone corticotropin-releasing hormone (CRH) is synthesized by human trophoblast, amnion, chorion, and decidual cells,[1] and placental CRH is secreted into maternal and fetal plasma during pregnancy.[2-5] Although CRH is present at extremely low levels in the plasma of nonpregnant adults, levels rise throughout the second and third trimesters, increasing exponentially in the final 6 weeks before parturition at term.[3,4,6] Concentrations of CRH messenger ribonucleic acid in the placenta parallel this well-described pattern of secretion.[7]

Evidence from in vitro and in vivo studies suggests that placental CRH is biologically active and stimulates the pituitary-adrenal axis during pregnancy.[3,8,9] In contrast to hypothalamic CRH, which is suppressed by glucocorticoids, secretion of CRH by trophoblast cells is stimulated by glucocorticoids in vitro,[10,11] suggesting a positive feedback between the fetal pituitary-adrenal axis and placental CRH. According to this model, progressive stimulation of all the compartments of the placental-pituitary-adrenal axis may culminate in parturition.[10]

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CRH release by trophoblast cells in culture is stimulated by oxytocin.[12] In addition, CRH increases prostaglandin E_2 and $F_{2\alpha}$ production by trophoblast and fetal membrane cells in vitro.[1] It has been shown that CRH increases the contractility of myometrial cells in the presence of oxytocin in vitro.[13] In view of these studies, some investigators have suggested a role for placental CRH in the mechanisms of human parturition.[10,12,13]

Because placental CRH may be involved in mechanisms leading to labor, we studied it in pregnancies complicated by preterm labor and during normal term labor. We hypothesized that the rise in maternal plasma CRH levels that precedes normal parturition at term occurs early in pregnancies complicated by preterm labor. The purpose of these studies was to determine whether (1) maternal plasma CRH levels are elevated during preterm labor, (2) levels are elevated when preterm labor is associated with infections, (3) levels are higher than normal before the onset of preterm labor, (4) levels remain elevated after suppression of uterine contractions with tocolytic agents, and (5) levels rise during normal term labor.

Material and methods

The end point for entry into the studies investigating maternal plasma CRH levels in association with preterm labor was identification of preterm labor rather than preterm delivery. We used criteria for the diagnosis of preterm labor as defined by Creasy,[14] including uterine contractions before 37 completed weeks of gestation in the presence of progressive cervical dilatation,

effacement, or rupture of membranes. Gestational age was determined for all fetuses by the history of the last menstrual period and first-trimester clinical examination. Confirmation by ultrasonographic examination was obtained for all patients diagnosed with preterm labor and in normal pregnancies as indicated. Treatment decisions were made by the patients' physicians in all cases and were not affected by participation in these studies. Signed informed consent was obtained from all subjects. These studies were approved by our institutional review board. In all studies a significance level of $p < 0.05$ was used. Data are presented as mean ± SEM.

Women admitted to the labor suite for treatment of preterm labor. We obtained a single venous blood sample from 39 women between 24 and 36 weeks of gestation admitted to our labor and delivery suite for treatment of preterm labor. In 34 cases blood samples were obtained before initiation of any medications. However, in five cases infusions of magnesium sulfate had been started before samples were obtained. The mean plasma CRH level in the women after magnesium infusion was not different from that in the rest of the women, and therefore these data were combined. In no cases were glucocorticoids given before blood samples were obtained.

The mean maternal plasma CRH concentration for women with preterm labor was compared with that for a group of normal nonlaboring pregnant women at the same gestational ages, by use of two-way analysis of variance.

Preterm labor associated with infections. All women admitted for treatment of preterm labor were evaluated for evidence of associated infections. In nine women admitted between 24 and 36 weeks of gestation, associated clinical infections were identified. These infections included chorioamnionitis and asymptomatic bacteriuria. Diagnosis of chorioamnionitis was made with previously described criteria, including maternal fever (≥38° C), maternal or fetal tachycardia, uterine tenderness, leukocytosis, and foul odor of the amniotic fluid, in the absence of other identified infection.[15] Placental pathologic studies were used when available to confirm the clinical diagnosis. Asymptomatic bacteriuria was diagnosed on the basis of a single urine culture with a colony count of 10^5/ml urine of a single organism.

The mean maternal plasma CRH levels for women with preterm labor both with and without infections were compared with the mean concentration for women with normal pregnancies by analysis of variance. The Student–Newman-Keuls post hoc test was applied to evaluate the differences in mean maternal plasma CRH levels between groups.

Women monitored prospectively for risk of preterm labor. To investigate maternal plasma CRH levels in women before preterm labor developed, we obtained venous blood samples from 17 women between 29 and 36 weeks of gestation who were identified to be at risk for preterm labor and referred to our high-risk antepartum clinic. Identified risks included previous premature delivery ($n = 15$), diagnosis of uterine didelphys ($n = 1$), and enlarging uterine myomas ($n = 1$). Eight women in the group subsequently had preterm labor, whereas nine had uncomplicated courses and were delivered at term. In some women we obtained more than one blood sample between 29 and 36 weeks of gestation. For statistical analysis, the mean CRH concentration of these multiple samples was used. The mean maternal plasma CRH levels for these groups and the mean level of CRH in women with normal pregnancies between 29 and 36 weeks of gestation were compared by analysis of variance with the Student–Newman-Keuls post hoc test of significance.

Women followed up after treatment for preterm labor. We studied 10 women who had been treated for preterm labor but who were not delivered prematurely. All of these women were being treated with oral terbutaline. Venous blood was obtained from these women at 29 to 36 weeks of gestation. The mean plasma CRH concentration for this group after preterm labor was compared with the mean level for the control group of women with normal pregnancy at the same gestational age by unpaired t test.

The effect of term labor on maternal plasma CRH levels. To study the effects of labor itself on maternal plasma CRH concentrations, we obtained venous blood samples from six women in both the latent phase (cervical dilatation <3 cm) and the active phase (dilatation >5 cm) of normal labor at 37 to 40 weeks of gestation. Mean maternal plasma CRH levels for each woman in latent and active labor were compared by paired t test.

Women with normal uncomplicated pregnancies. Venous blood samples were obtained from 30 women monitored in our routine antepartum clinics between 24 and 36 weeks of gestation. These women had normal uncomplicated pregnancies and subsequently were delivered at term.

CRH radioimmunoassay. Blood was collected in tubes containing ethylenediaminetetraacetic acid (1.5 mg/ml), chilled immediately, and centrifuged within 8 hours of sampling. Plasma was frozen at −20° C and thawed once for assay. CRH was extracted from plasma with Sep Pak C-18 cartridges (Waters Associates, Milford, Mass.), and CRH concentrations were determined by radioimmunoassay with an antihuman CRH antibody raised in our laboratory and human CRH standard (Peninsula Laboratories, Inc., Belmont, Calif.).[2] The mean recovery of cold CRH added to charcoal-stripped plasma is 65%.

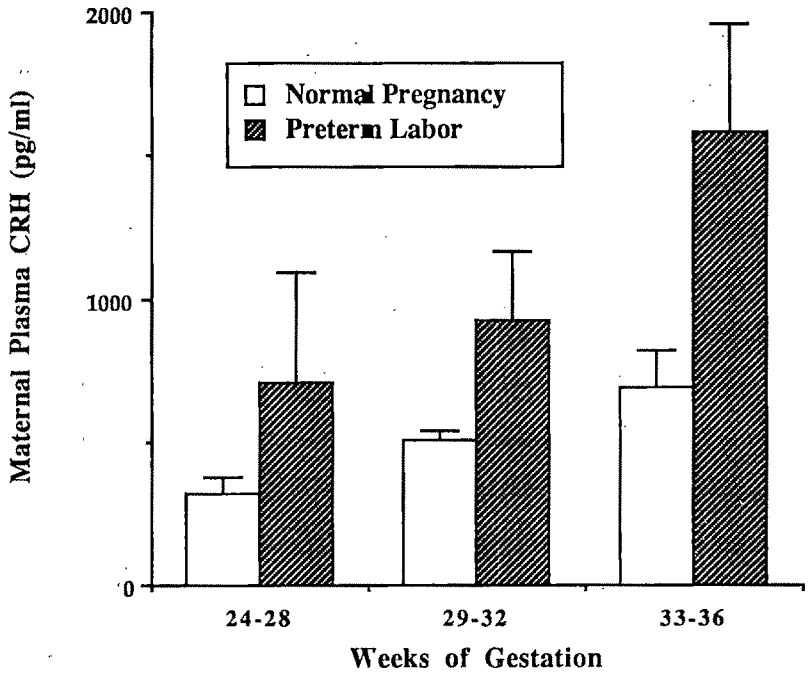


Fig. 1. Mean maternal plasma CRH levels (picograms per milliliter) in two study groups, women admitted for preterm labor and women with normal pregnancies not in labor in three gestational age groups. By two-way analysis of variance, there was significant main effect of study group on mean maternal plasma CRH levels [$F_{5.21}$ (1, 69), $p < 0.05$] with higher mean maternal plasma CRH levels in women with preterm labor.

Table I. Maternal plasma CRH mean ± SEM, in women admitted to our labor and delivery suite for treatment of preterm labor and in normal pregnancy

	Normal pregnancy		*Preterm labor*	
Gestational age (wk)	*CRH level (pg/ml)*	*No.*	*CRH level (pg/ml)*	*No.*
24-28	325 ± 47	12	772 ± 440	7
29-32	505 ± 110	9	913 ± 200	17
33-36	692 ± 125	9	1610 ± 410	15

Results

Women admitted to labor suite for treatment of preterm labor (Fig. 1). In the 39 women studied on admission to our labor suite for treatment of preterm labor, 14 were treated with tocolytic therapy and their uterine contractions were suppressed. All these women were discharged from the hospital on a regimen of oral ritodrine or terbutaline therapy. Ten subsequently were delivered at term, whereas four were lost to follow-up. For 25 women uterine contractions could not be suppressed or tocolytic therapy was contraindicated, and all of these women were delivered of preterm infants. The mean maternal plasma CRH levels were similar for women with preterm deliveries and those who were treated for preterm labor and discharged undelivered.

The mean maternal plasma CRH levels for all 39 women admitted in preterm labor and for women with normal pregnancies are shown in Fig. 1. The descriptive statistical data are also shown in Table I. Overall, the mean maternal plasma CRH concentration was higher in the preterm labor group than in normal pregnancy by two-way analysis of variance ($F = 5.21$, $p < 0.05$).

Preterm labor associated with infections (Fig. 2). Nine of the women admitted in preterm labor between 24 and 36 weeks of gestation had the clinical diagnosis of chorioamnionitis or asymptomatic bacteriuria in association with preterm labor. As shown in Fig. 2, women with preterm labor and associated infections had a mean maternal plasma CRH level similar to that of women with normal pregnancy (660 ± 104 and 490 ± 50 pg/ml, respectively). At the same gestational ages, women with preterm labor and no infections had a significantly higher mean maternal plasma CRH

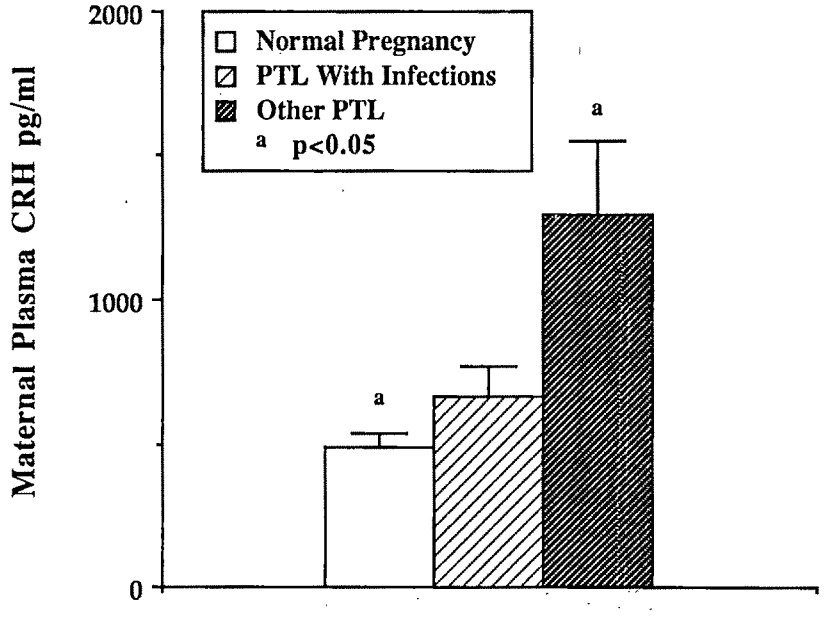


Fig. 2. Mean maternal plasma CRH levels in women at 24 to 36 weeks of gestation in preterm labor without associated infections *(Other PTL)*, in preterm labor associated with clinical infections *(PTL With Infections)*, and in normal pregnancy. By analysis of variance there was a significant effect of study group on maternal plasma CRH level [$F_{6.83}$ (2, 66), $p < 0.005$]. Mean maternal plasma CRH level for women with preterm labor and no associated infections was significantly higher than in normal pregnancy ($p < 0.05$). There was no difference in mean CRH concentration for women with infections and in normal pregnancy.

level, 1220 ± 170 pg/ml, than in normal pregnancy ($p < 0.05$).

Women followed up prospectively for risk of preterm labor (Fig. 3). We next examined the maternal plasma CRH levels in 17 women followed up in our high-risk antepartum clinic because they were at risk for preterm labor. Although nine of these women with follow-up at 29 to 36 weeks of gestation did not have preterm labor and subsequently were delivered at term, 8 of the 17 high-risk women had preterm labor. The mean maternal plasma CRH concentrations for these women and for women with normal pregnancy at 29 to 36 weeks of gestation are shown in Fig. 3. The mean maternal plasma CRH concentration was 990 ± 140 pg/ml before the onset of preterm labor. This was significantly higher than the CRH concentration in women at risk who did not have preterm labor, 560 ± 120 pg/ml, and also for women with normal pregnancy at the same gestational age, 600 ± 67 pg/ml ($p < 0.05$).

Women after treatment for preterm labor (Fig. 4). Ten women were referred to the high-risk antenatal clinic after they were treated for preterm labor. The mean maternal plasma CRH levels for these women was compared with the mean concentration for women during uncomplicated pregnancy at 29 to 36 weeks of gestation. The mean maternal CRH concentration in women after treatment for preterm labor, 1240 ± 320 pg/ml, was higher than in normal pregnancy at the same gestational age, 600 ± 67 pg/ml ($p < 0.05$).

The effect of term labor on maternal plasma CRH levels (Fig. 5). To evaluate the effect of labor itself on maternal plasma CRH levels, we compared CRH concentrations in latent and active labor in six women at term. The mean maternal plasma CRH concentration in latent labor, 1560 ± 180 pg/ml, was not significantly different from the mean concentration in active labor, 1650 ± 360 pg/ml.

Comment

In this study we report an association between early elevation of maternal plasma CRH levels and preterm labor. We studied women admitted to our labor suite for treatment of preterm labor and found significantly higher maternal plasma CRH levels in women with preterm labor than in those with normal pregnancy. However, in a subgroup of this population, women with preterm labor and associated infections, there was no elevation of maternal plasma CRH compared with levels in normal pregnancy. We also found high CRH levels before the clinical diagnosis of preterm labor in women at risk of preterm delivery. After treatment of preterm labor and suppression of uterine contractions, maternal plasma CRH levels were also higher than the levels seen in normal uncomplicated pregnancy. When we examined the effect of term labor on maternal

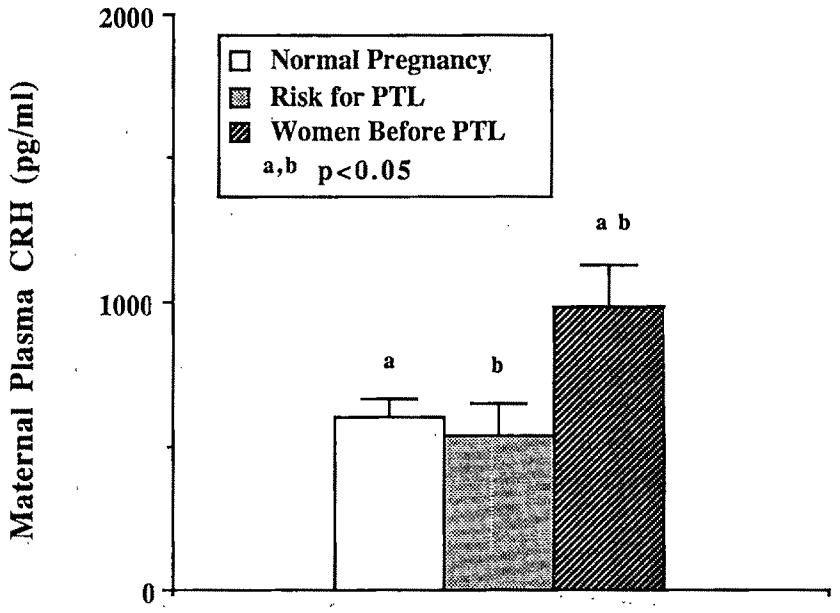


Fig. 3. Mean maternal plasma CRH levels in women monitored prospectively for risk of preterm labor and in normal pregnancy. Women at risk for preterm labor were divided into two groups: those in whom preterm labor ultimately developed *(Women Before PTL)* and women who were at risk but who did not have preterm labor *(Risk for PTL)*. By analysis of variance there was a significant effect of study group on maternal plasma CRH concentration [$F_{4,78}$ (2, 32), $p < 0.02$]. Women in whom preterm labor subsequently developed had higher mean maternal plasma CRH level than either women with normal pregnancy or women at risk who did not have preterm labor ($p < 0.05$). *PTL*, preterm labor.

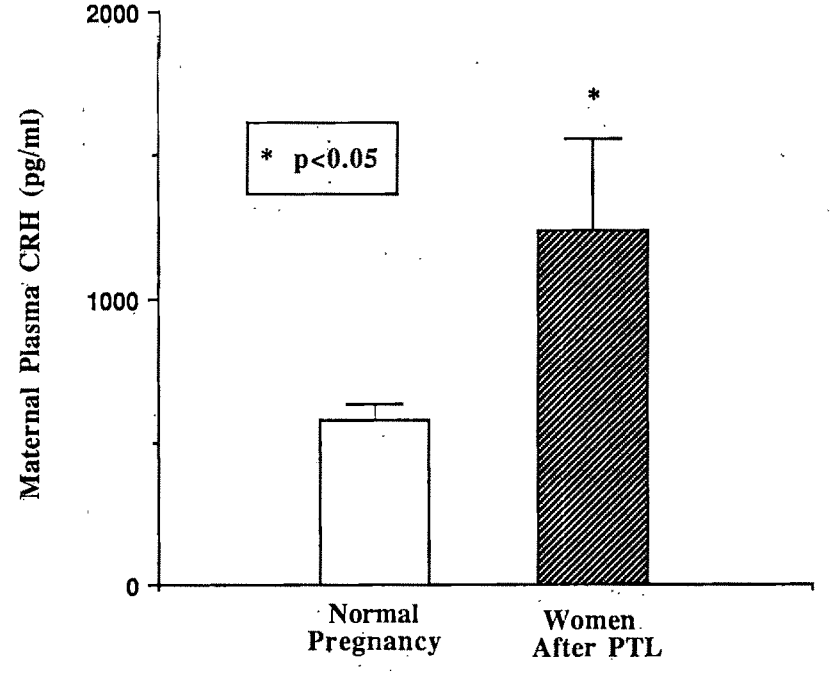


Fig. 4. Mean maternal plasma CRH levels at 29 to 36 weeks of gestation in women after treatment for preterm labor and in normal pregnancy Mean maternal CRH concentration was significantly higher for women after treatment than for women with normal pregnancies by unpaired t test ($p < 0.05$). *PTL*, preterm labor.

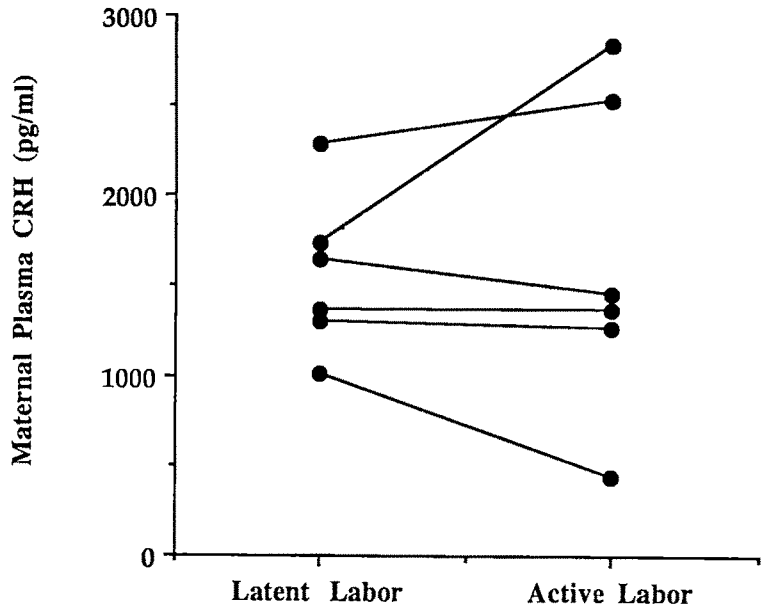


Fig. 5. Maternal plasma CRH levels in six women during both latent and active labor at 37 to 40 weeks of gestation. Mean plasma CRH levels were not different in latent and active labor.

plasma CRH levels, we found no change in mean plasma CRH levels during the acute stress of labor.

Some investigators have reported rising CRH levels during normal labor at term, suggesting that processes during labor may cause maternal plasma CRH to increase.[5, 16, 17] In one study initial blood samples were obtained as much as a week before the onset of labor.[16] Maternal plasma CRH concentrations rise exponentially in the weeks before parturition, and therefore the reported increase in CRH levels may have occurred before labor began. In another study samples in latent and active labor were obtained from different women.[5] Variation between these groups could account for the differences reported. Other investigators have found no significant increase in maternal plasma CRH levels during labor.[4, 6, 13] We found no increase in maternal CRH levels in active labor as compared with latent labor in women at term. Furthermore, maternal plasma CRH levels were elevated before labor was diagnosed in women who ultimately had preterm labor. This suggests that elevated CRH levels in patients who had preterm labor were due to mechanisms that began before the onset of labor and were not due to the process of labor itself.

Women followed up in the high-risk clinic after treatment for preterm labor had higher plasma CRH levels than found in normal pregnancy. Therefore suppression of uterine contractions at the myometrial level by tocolytic agents did not appear to cause a corresponding suppression of placental CRH secretion, although a stimulatory effect of these agents on placental CRH secretion cannot be excluded. The continued elevation of maternal plasma CRH levels without clinical evidence of uterine contractions suggests that, although activated placental CRH secretion may play a role in the initiation of parturition, it is not sufficient to cause labor and that other factors are necessary for labor to occur.

We did not use premature delivery as the end point for this study because the success of treatment for preterm labor depends on many factors unrelated to the mechanisms of labor. These include the lag time between onset of contractions and presentation to the labor suite, association with spontaneous rupture of membranes, and other perinatal complications. Further studies are needed to determine if our ability to suppress uterine contractions with tocolytic agents was related to the maternal plasma CRH concentration.

Preterm labor represents a clinical end point that most likely can be reached through several mechanisms. Studies have shown an association between preterm labor and infectious processes.[19-22] Pathways to the stimulation of uterine contractions have been suggested through bacterial endotoxins and release of cytokines in response to infectious processes, resulting in increased prostaglandin production in the amnion and decidua.[23-25] We report that maternal plasma CRH levels in women with infections and preterm labor were similar to levels found in normal pregnancy. However, CRH concentrations in women in preterm labor with-

out infections were significantly higher than normal. We thus may have identified a subset of women with preterm labor in whom secretion of placental CRH is abnormally activated. In these women the pathway to preterm labor may be through paracrine or endocrine mechanisms involving the placental-pituitary-adrenal axis.

In summary, we have shown that maternal plasma levels of placental CRH are elevated early in pregnancies complicated by preterm labor. This does not appear to be related to the process of labor itself as high levels are found before preterm labor is diagnosed clinically and we found no rise in maternal CRH levels during term labor. In a subset of women with preterm labor and associated infections, plasma CRH levels were similar to levels seen in normal pregnancy. These data suggest that early activation of the placental-pituitary-adrenal axis, as reflected by early elevations in maternal plasma CRH concentrations, may be part of a cascade of events leading to the onset of premature labor in some pregnancies.

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Discussion

Dr. Atef Moawad, Chicago, Illinois. The etiology of the initiation of term and preterm labor in women is still, to a great extent, unknown. We have witnessed over the years the debates concerning many theories—to mention some, the oxytocin theory championed by Caldeyro-Barcia, the placental progesterone theory and its local blockade to the myometrium by the late Arpad Csapo, and then there was a great excitement when Liggins identified the important role of the fetal adrenal cortex in initiating labor in the sheep. However, the effect of cortisol in the human in initiating labor, to date, remains doubtful.

We know that cortisol and cortisol-binding globulin

levels increase substantially in pregnancy,[1] and so does free and bound cortisol.[2] We know also that maternal CRH has been shown to be of placental origin. The placenta has a higher concentration of it,[3] its progressive rise in pregnancy coincides with placental maturation, and it is significantly higher in multiple pregnancies. We also know that CRH has no effect on corticotropin and cortisol secretion in women in late pregnancy.[4]

The authors are to be commended for undertaking a difficult study in which they measured CRH in pregnant women and in those who had normal labor at term. These were compared with those at risk for preterm labor. The latter group was prospectively followed up to the final outcome of either preterm labor or uncomplicated term pregnancy. They further broke down the preterm labor group into infected and uninfected cases.

Their results indicate that CRH is higher in laboring women when compared with those not in labor. They thus confirmed previous reports in which it has been shown that this hormone progressively increases in maternal plasma during pregnancy, increases further during delivery, and declines rapidly to nonpregnant levels on the first day post partum.[5-8]

The authors report the significant finding that the elevation of the maternal serum CRH precedes the onset of preterm labor (in those women at risk who eventually had progression to premature labor). To our surprise this is not the case in those patients who had an infectious process that presumably contributed to the initiation of preterm labor.

These results, again, prospectively confirm what Campbell et al.[6] showed retrospectively, that women with premature labor had plasma CRH concentrations higher than those of women with normal labor for a substantial period of time before the onset of labor.

The lack of rise of CRH in cases with infections is somewhat surprising. The authors did not elaborate on mechanisms that may be implicated. Are prostaglandins implicated as a common factor that may regulate placental CRH release? If so, why shouldn't CRH be elevated in cases of chorioamnionitis? To study this process in detail one must have more data concerning the organisms involved and the breakdown of these cases. Another question that needs to be asked is, can asymptomatic bacteriuria be considered as an infection?

One must also ask the question whether it is valid to use a single venous sample for determinations from patients with gestational ages between 24 and 36 weeks. It is expected that there would be considerable variations among individual women. Consequently, the statistics based on population or group comparison are not very convincing. It would be necessary to monitor several women longitudinally with blood samples in a serial fashion at close intervals. Some of these data have already been reported by Campbell et al.[6] One needs to know if there are diurnal variations or pulsatile secretion of the hormone.

Preterm delivery was not used as an end point. The reason given is the presence of many factors unrelated to the mechanisms of labor. Still, one would be interested in the final outcome, especially in the infected group. Although some of the data are combined appropriately in small gestational age groups (e.g., the effects of gestational age or CRH), other data are pooled from women at 29 to 36 weeks. On the basis of observed significant effects of gestational age on CRH, the appropriateness of pooling data over such an extended period of gestation is unclear.

In summary, the authors confirmed previous findings of the progressive increase in maternal CRH in pregnancy and its further rise in labor, with significantly higher levels in cases with preterm labor not associated with infections.

I commend the authors for undertaking a difficult study with many variables. I have some concerns about the pooling of the data and the lack of multiple longitudinal observations, especially in the cases that were prospectively followed up; perhaps this becomes a more compelling argument when the number of cases in some of the studied groups was small. One must consider this report preliminary and I would encourage Dr. Warren to continue the study further to validate these data.

We know from previous studies that there are low CRH levels in umbilical cord plasma, which implies that there is limited or no transfer of the placental CRH to the fetus. This means that the role of the fetus in this process is minimal. Where does this leave us as far as the fetal adrenal secretion and the mechanisms of parturition?

I wish to congratulate the authors and hope that they further elucidate some of the mechanisms that explain their results and delineate a role for this hormone.

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DR. STEPHEN L. CURRY, Hartford, Connecticut. A second major problem for the practicing obstetrician is that of postdate pregnancy. I wonder if you looked at those patients who have postdates pregnancies, and do they have lower levels of CRH compared with normal patients?

DR. EBERHARD MUELLER-HEUBACH, Winston-Salem, North Carolina. With regard to chorioamnionitis, how many of the nine patients had chorioamnionitis compared with asymptomatic bacteriuria, and were there any placental pathology results available to indicate whether there was histologic chorioamnionitis? As I understand it, these were cases of clinical chorioamnionitis.

Furthermore, of the eight women who went into preterm labor out of the 17 at high risk of preterm labor, how many of those actually went on to delivery and were there any differences in CRH level among those who were delivered versus those in whom the preterm labor was successfully inhibited?

DR. CALVIN J. HOBEL, Los Angeles, California. If stress is an important part of preterm labor, i.e., the central nervous system–adrenal axis, then this could possibly mean that uteroplacental stress could increase CRH, and the question would be, what is the stimulus? Have you looked carefully at histories of these patients to sort out mental stress that would involve the maternal corticoadrenal axis or physical stress that would sort out peripheral release of neurotransmitters?

DR. JOHN LARSEN, Washington, D.C. If these were cases of asymptomatic chorioamnionitis, what method was used for sorting them out? Was it strictly cultures and maternal serologic testing or was there any invasive testing such as amniocentesis?

DR. ROBERT K. CREASY, Houston, Texas. I have two questions relative to the potential usefulness of this test. It would appear that you were giving standard errors and that these were relatively large. Could you give us some indication as to whether the ranges are going to be so wide as to potentially be useful in the clinical setting?

And second, in the patients who had elevations that were being monitored antenatally and who subsequently did have preterm labor, can you give us some idea as to the time interval between blood sampling and the development of the preterm labor process?

DR. MICHAEL R. SOULES, Seattle, Washington. From an endocrinology point of view, it has been known that measuring hypothalamic neuropeptides in peripheral serum is difficult, so I would like to know more about the CRH assay that you were using.

DR. WARREN (Closing). Let me address that last question first. We collect blood in chilled tubes, spin the blood down, and freeze the plasma. We extract the CRH with Sep Pak C-18 cartridges, after which plasma is assayed with our own antihuman CRH antibody. The intraassay coefficient of variation is 6.3%; our interassay variation is about 17%. Our recovery of synthetic human CRH is about 65%. This assay was developed by Dr. Goland in our laboratory and has been very consistent over the last 4 years or so.

To address the issue of elevation of CRH from labor itself, it is still controversial. A number of investigators have shown that CRH does increase during labor, whereas others have shown that it does not. In a preliminary study we looked at six women in latent and active labor and found no difference between levels in these women with a paired *t* test.

To address your questions on chorioamnionitis and infection, we used clinical criteria for infection and we grouped chorioamnionitis and asymptomatic bacteria together. We used a clinical diagnosis for chorioamnionitis as described by Gibbs. When we had pathologic specimens of the placentas, we used them to correlate with the clinical diagnosis. We did not have pathologic studies on all the patients. Therefore we are continuing the study now, looking at histologic diagnosis of chorioamnionitis and measuring levels of the peptide in maternal plasma and in placental extracts.

To address some of the questions with regard to the cross-sectional aspect of the study, our numbers were too small to describe the interval between sampling for CRH and the onset of preterm labor. However, we are now starting a study in which we measure levels sequentially in women at risk for preterm labor to begin to get an idea of the actual curve of the CRH in the plasma. In other words, is the curve actually shifted over so that it increases gradually to a certain point at which labor occurs or is there a sudden increase?

Our group has shown that CRH is secreted in fairly constant levels throughout the day, so there does not appear to be diurnal variation. It does not appear to be pulsatile, although we have not done the studies—and I do not believe anyone has done the studies with frequent enough measurements to determine whether it is secreted in a pulsatile nature.

As far as the question about pooled data, we obviously were very concerned that differences in gestational age would influence the level of CRH because levels go up so dramatically with gestational age, especially near term. We analyzed the gestational ages in the different groups of pooled data, and they were virtually identical with no statistical difference. So, although these are pooled data and it would be nice to have a week-by-week sample, in these small numbers we were not able to do that analysis.

CRH is present in the umbilical cord at lower levels than in the maternal plasma, and as I said, umbilical venous levels are higher than umbilical arterial levels, also suggesting the CRH is coming from the placenta. The levels are certainly within the biologically active range in the fetal plasma.

We have looked at a few patients who are postdates, but as you know from the collaborative study it is very

hard to confirm that those patients are postdates. It is something we would like to look at, but we have not gotten to it just yet.

We did break down our data to determine how many women were delivered preterm and how many were not and how many were successfully treated with tocolytic agents. There were no significant differences in CRH levels between those two groups. However, I have to say that our numbers are too small and we would like to look at that question in larger numbers to see if the actual level of CRH was related to the success or failure of tocolytic treatment.

As far as intrauterine stress and CRH, we do not know what regulates placental CRH in vivo. We know that stress certainly stimulates hypothalamic CRH, but the placenta is a completely different structure and the regulation of CRH in the placenta is unclear as yet. So I do not think that we can say that CRH is released by the placenta in acute stress. The role of chronic stress is under investigation.

As far as the potential usefulness of this as a predictor for preterm labor, the variation of levels between patients and even among single patients is quite large. In addition, women can have fairly high levels without the onset of labor, and women can have very low levels and develop labor. So I do not think we can really consider this yet as a clinical tool.

Clinical Articles

Rates and outcomes of planned pregnancy after use of Norplant capsules, Norplant II rods, or levonorgestrel-releasing or copper TCu 380Ag intrauterine contraceptive devices

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New York, New York, Santiago, Chile, Santo Domingo, Dominican Republic, Los Angeles and San Francisco, California, Singapore, Jyväskylä, Finland, and Uppsala, Sweden

OBJECTIVES: The objectives were to measure rates of planned pregnancy and factors affecting these rates after use of very-long-acting contraceptive methods (Norplant or Norplant II implants, a levonorgestrel-releasing intrauterine contraceptive device, or the copper T (model TCu 380Ag) intrauterine contraceptive device.

STUDY DESIGN: The design was a concurrent, multicenter prospective study of 372 women who stopped contraception for planned pregnancy. Analysis was by life-table, log-rank, and standard χ^2 methods.

RESULTS: Pregnancy rates 12 and 24 months after cessation of contraceptive use were 82 and 89 per 100, respectively. Age at removal and family planning intentions at initiation of contraception were both significantly correlated with pregnancy rates ($p < 0.05$), but former contraceptive regimen and duration of contraceptive use were not. Pregnancy outcomes did not deviate from normal limits and did not differ by contraceptive formerly used.

CONCLUSION: Contraceptive implants releasing 30 μg/day of levonorgestrel or intrauterine contraceptive devices releasing 20 μg/day of levonorgestrel or having copper surface areas of 380 mm² are associated with normal fertility after use for women seeking pregnancy at termination. Durations of use do not affect pregnancy rates. (Am J Obstet Gynecol 1992;166:1208-13.)

Key words: Pregnancy after contraception, implant reversibility, post-IUD fertility

Preservation of reproductive potential is an essential feature of contraceptive methods. Studies have shown an initial delay of fertility after combined oral contraceptive use in comparison with the experience of women who stopped use of intrauterine contraceptive devices (IUDs) or diaphragms.[1] The progestin-only injectables, depot medroxyprogesterone acetate and norethindrone acetate, also have been associated with delays of pregnancy subsequent to use.[2-4] Proportions pregnant within 12 or 24 months, however, indicate normal fertility after cessation of use of these steroidal contraceptives. In contrast, case-control studies have suggested an association between prior use of IUDs and infertility at 1 year, mediated by pelvic inflammatory disease and multiple sex partners.[5, 6] Because preservation of reproductive potential is a critical issue in contraception, as new contraceptives emerge and progress toward general distribution, they require evaluation of the pace and completeness of achievement of pregnancy and childbirth after cessation of method use. The need for evaluation is particularly compelling with very-long-acting medicated contraceptive methods. Prolonged medication may affect normal reproductive

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function even after contraception has ceased and may therefore impose extended delays in conception. We report pregnancy rates and outcomes subsequent to cessation of use of four relatively new, long-acting contraceptives, all of which first received regulatory approval between 1983 and 1990 for periods of 3 to 5 years of continuous use.

Material and methods

Contraceptive methods. Two medicated IUDs, a device releasing 20 μg/day of levonorgestrel and a copper T, model TCu 380Ag, were in randomized trials from 1981 through 1990 in seven centers.[7-9] Another set of seven clinics compared two subdermal implant systems, Norplant capsules and Norplant II rods, in trials from 1982 through 1990.[10-12] Clinics participating in both trials contributed 76% of studied removals for planned pregnancy.

The Norplant capsule system (Leiras Pharmaceuticals, Turku, Finland) releases a rated 30 μg/day of levonorgestrel for 5 years. After removal of capsules, residual levonorgestrel is rapidly metabolized. With an initial half-life of 18 hours, the drug is undetectable 1 week after removal.[13]

The Norplant II system (Leiras) is a set of two 4.4 cm rods that release 30 μg/day of levonorgestrel from a cured homogeneous mixture of the drug and a polydimethylsiloxane elastomer covered by thin-walled silicone rubber tubing. Once these rods are removed, levonorgestrel levels decrease as described for capsule implants. Rod implants have been approved for 3 years of continuous use in Finland. Subjects assigned to particular Norplant II manufacturing lots were, however, permitted continuous use for 5 years.

The levonorgestrel-releasing IUD (Leiras) releases drug at a rated 20 μg/day from a cured homogeneous rod of levonorgestrel and polydimethylsiloxane elastomer. Two variants with drug loads of 46 and 60 mg were studied for 5 and 7 years, respectively. The IUD, here designated as the LNg 20, has been approved for 5 years of use in Finland.

The copper T IUD, model TCu 380Ag (Outokumpu Oy, Pori, Finland), has a copper surface of approximately 380 mm^2. A silver core wire differentiates this from the similar device marketed in the United States. Approved for 5 years of use in Finland, this IUD was studied for 8 years of continuous use.

All four contraceptive systems were developed by the Population Council's International Committee for Contraception Research or by the Population Council's licensee.

Conduct of trial and follow-up after removal. For admission to either trial, women had to be healthy, to be regularly exposed to the risk of pregnancy, and to have no contraindications to combined oral contraceptives; in the IUD trial they had to be without uterine anomalies contraindicating IUD use. Previous pregnancy was required. Women with a history of pelvic inflammatory disease since the last pregnancy were excluded. At admission, women in the IUD trial were 18 to 38 years old; in the implant study they were 18 to 40 years old.

Subjects gave informed consent and were monitored 1, 3, and 6 months after admission and semiannually thereafter. Except in the case of IUD expulsion, stopping required a clinic visit for extraction of the IUD or implant. Three-year continuation rates were >50 per 100 for each regimen. Large percentages of subjects using Norplant, Norplant II, or the levonorgestrel-releasing IUD therefore reached the putative end of the contraceptive's effective period. The majority of these women adopted contraception again immediately after removal, as had the majority of subjects who discontinued use because of menstrual or other medical problems.

Women with removals for planned pregnancy were asked to maintain contact until pregnancy and its subsequent outcome. Data in this report stem from clinics that determined the subsequent pregnancy status of at least 75% of subjects who ceased use by October 1990. As of July 1991, 91.9% of these women had been contacted after removal for planned pregnancy.

Rates of planned pregnancy were computed by actuarial life-table methods, which calculate the exposure interval from the date of removal of the contraceptive device to the date of pregnancy, or report of change of mind, or separation from husband or partner, or use of another contraceptive. After removal, between 8% (LNg 20 IUD) and 19% (Norplant) of women changed their minds and/or were no longer exposed to the risk of pregnancy. These women are included during the exposure interval in the analysis of pregnancy rates. Eight percent of women were not seen after removal and are not included in the life tables. Log-rank χ^2 methods tested statistical significance among life-table pregnancy rates. Contingency table χ^2 methods tested for statistically significant differences among pregnancy outcomes and subject characteristics.

Characteristics of subjects. At seven clinics 2748 subjects had enrolled in contraceptive trials. By October 1990, 372 of these requested removal for a planned pregnancy. Smaller proportions of implant subjects (10% to 12%) than of IUD subjects (17% to 20%) sought such a removal (Table I). Median ages at admission were 26 years for all IUD subjects and 27 years for the implant subjects. Average age at admission of the women who later sought planned pregnancy was 1 to 2 years below the overall average. Mean age at removal for planned pregnancy was 27.8 years, with significant variation by contraceptive method (Table I) ($p < 0.05$).

Table I. Characteristics of women planning pregnancies by contraceptive method used

Characteristic and category	*Contraceptive method used*			
	LNg 20 (n = 91)	*TCu 380Ag (n = 103)*	*Norplant (n = 62)*	*Norplant II (n = 116)*
Age at device removal (yr)				
<30	69	66	63	78
≥30	31	34	37	22
Mean ± SE	27.8 ± 0.4	28.6 ± 0.4	28.2 ± 0.5	26.7 ± 0.3
Parity				
0 or 1	46	50	48	47
≥2	54	50	52	53
Mean ± SE	1.75 ± 0.09	1.70 ± 0.09	1.71 ± 0.14	1.80 ± 0.10
Family planning intention				
Spacing	66	66	50	61
Limiting	24	23	29	22
Uncertain	10	11	21	17
Duration of method use (mo)				
<24	41	31	29	38
25-48	43	36	52	58
49-100	16	33	19	4
Mean ± SE	30.2 ± 1.7	38.9 ± 2.2	33.6 ± 1.9	29.1 ± 1.2
History of pelvic inflammatory disease				
No	87	84	NA	NA
Yes*	13	16	NA	NA
At clinic common to both studies				
Yes	80	81	72	70
No	20	19	28	30

Values are numbers of women per 100. *NA,* Not available.
*History of pelvic inflammatory disease before last pregnancy.

Women who had used Norplant II implants were youngest at removal, 26.7 years, while copper T users were oldest, 28.6 years. Age at Norplant capsule removal was 1.5 years greater than age at removal of Norplant II ($p < 0.05$) (Table I).

Mean parity at removal was 1.75, with a range from 1.70 to 1.80 children by contraceptive method (Table I). Twenty-four percent of women requesting removal for planned pregnancy had stated at admission they did not want additional children.

Women planning pregnancy used the copper T device an average of 38.9 or 8.7 months longer than their peers used the LNg 20 IUD ($p < 0.01$) (Table I). Norplant capsules were used a mean of 4.5 months longer than were rod implants ($p < 0.05$).

Results

For the entire group of women who planned pregnancy, the 12-month life-table rate of pregnancy was 82 per 100. This rate ranged by device from 77 to 84 per 100 (Table II). Two years after removal, the pregnancy rate was 89 per 100, varying by former contraceptive regimen from 87 to 92 per 100 (Table III). Neither 1- nor 2-year cumulative conception rates differed significantly by method formerly used.

Eighty-five per 100 women seeking pregnancy before age 30 achieved it in the first year, a rate not significantly affected by method formerly used (Table II). When the quest for pregnancy began at age ≥30, the 1-year pregnancy rate was about 10 per 100 lower than that of women aged <30 ($p = 0.08$). Higher pregnancy rates among younger women were manifest with each of the four methods (Table II). Two years after ceasing contraception, 93 per 100 of the younger women were pregnant, a rate 13 per 100 higher than that of women ≥30 years old ($p = 0.03$, Table III).

Univariate analysis by duration of contraception indicated no significant variation in pregnancy rates ($p = 0.20$). Among women who had used contraception ≥3 years, the 12-month pregnancy rate was 84 per 100 and was essentially uniform by method, 82 to 85 per 100 (Table II). Women who used contraception <3 years had a similar 12-month pregnancy rate, 81 per 100. Two years after removal, the pregnancy rate was 89 per 100 for each duration-of-use group.

Parity had little effect on conception rates. At 1 year the pregnancy rate of women with fewer than two children was 83 per 100, comparable to the 81 per 100 among women with two or more children (Table II). Two years after device removal, pregnancy rates by parity group were 89 to 90 per 100.

Family planning intention at the beginning of the

Table II. One-year life-table pregnancy rates per 100 after contraception

Item and category	*LNg 20 IUD*	*TCu 380Ag IUD*	*Norplant capsules*	*Norplant II rods*	*Total*	*Probability*	
						Between devices	*Between categories*
All women	84	77	83	84	82	0.09	NC
Age at removal (yr)							
<30	89	79	86	85	85	0.35	0.08
≥30	73	73	76	80	75	0.23	
Duration of use (mo)							
<37	85	72	84	84	81	0.18	0.20
≥37	82	85	83	85	84	0.38	
Parity							
0 or 1	81	79	81	90	83	0.10	0.96
≥2	87	73	100	79	81	0.57	
Desire for more children at admission							
Yes	85	86	84	86	85	0.17	0.03
No	81	57	68	73	70	0.12	
Uncertain	89	60	100	88	85	0.35	
History of pelvic inflammatory disease							
No	85	74	NA	NA	77	0.77	0.64
Yes*	79	100	NA	NA	88	0.09	
Duration of amenorrhea before removal (days)							
<30	83	76	79	87	NC	0.03	NC
30-89	80	†	†	80	NC	0.86	NC
≥90	92	†	†	†	NC	0.83	NC

NA, Not applicable; *NC*, not calculated.
*Women had been pregnant after pelvic inflammatory disease.
†Size of group too small for analysis.

contraceptive trial was significantly related to posttrial pregnancy rates. Women who had expressed a desire to have more children at study entry, i.e., contraceptive spacers, had pregnancy rates of 85 per 100 at 1 year and 92 per 100 at 2 years. These were substantially above the rates of 70 and 81 per 100 at 1 and 2 years, respectively, for family limiters, women who had stated at admission they did not want additional children ($p = 0.03$).

We examined effects on pregnancy rates of the dichotomous categories of age (<30 or ≥30 years), duration of contraceptive use (<37 or ≥37 months), and family planning intention at admission (spacer or limiter) by holding the other variables constant. Younger age (<30 years) was associated with higher pregnancy rates than was older age in all four duration–family planning intention groupings, confirming the significant finding at 2 years in the univariate analysis (Table III). Family planning spacers in all age–duration-of-use comparisons had higher pregnancy rates 1 and 2 years after removal than did women who entered the trials as limiters of family size. Women with longer durations of contraceptive use had higher pregnancy rates than did shorter-term users in all four age–family planning intention groups. The magnitudes of the effect of age and duration were greater among women who were initial limiters than among initial spacers.

The joint effects of family planning intention, age, and duration of use were strong. Women <30 years old at removal of the contraceptive who had used contraception to space children and who had used the contraceptive ≥37 months had a 1-year pregnancy rate of 93 per 100. In contrast, women ≥30 years old who used contraception to limit family size and used the contraceptive method <37 months had a pregnancy rate of 58 per 100 ($p < 0.02$).

Table III. Two-year life-table pregnancy rates after contraception

Category	*Rate per 100*	*Probability between categories*
All women	89	NA
LNg 20 IUD	88	0.07
TCu 380Ag	88	
Norplant capsules	87	
Norplant II rods	92	
Age		
<30	93	0.03
≥30	80	
Duration of use (mo)		
<37	89	0.26
≥37	89	
Parity		
0 or 1	89	0.99
2+	90	
Desire more children at admission		
Yes	92	0.03
No	81	

NA, Not applicable.

Table IV. Pregnancy outcomes: Percentage distribution

Outcome	*LNg IUD*	*TCu 380*	*Norplant*	*Norplant II*	*All methods*
Term delivery	89	82	93	88	87.1
Premature delivery	2	[illegible]	0	1	0.9
Spontaneous abortion	9	[illegible]	4	8	9.4
Elective abortion	0	[illegible]	4	2	2.1
Ectopic pregnancy	0	0	0	1	0.4
Total with known outcome	100	100	100	100	100.0
Known outcomes (% of all pregnancies)	96	91	85	93	92.1
No. pregnant	68	65	33	86	253

$p > 0.05$.

Women who reported a history of pelvic inflammatory disease followed by a pregnancy and then entry into the randomized IUD trial had the same rate of conception as did women with no reported history of PID (Table II).

Although conception rates 1 and 2 years after removal were within normal limits, pregnancy rates in the first 3 months after removal differed considerably among methods ($p < 0.01$). Fifty-eight percent of women who had discontinued Norplant II rods became pregnant within 3 months, in contrast to 34% of former users of levonorgestrel-releasing IUDs. Forty-three percent and 37%, respectively, of copper T IUD and Norplant capsule users were pregnant within 3 months.

During use of the levonorgestrel IUD, substantial proportions of women experienced no menstrual events in any given calendar month.[7, 8] When the levonorgestrel IUD was removed for planned pregnancy, 25% of women had not menstruated in the preceding 90 days. The first-month pregnancy rate was 5 per 100 for this group. First-month pregnancy rates for other users of this IUD also were below the expected range of 16 to 33 per 100. Nevertheless, by the end of the first year, cumulative conception rates by duration of amenorrhea preceding removal were in the normal range, 80 to 92 per 100 among former users of the levonorgestrel IUD (Table II).

Pregnancy outcome. By July 1991 the outcomes of 92.1% of the pregnancies had been established. Of these, 88% had terminated with a live birth. Two infants (0.9%) had birth weights <2500 gm (Table IV). One tubal pregnancy occurred in a former Norplant II rod implant user. Female infants constituted 50.5% of all infants born; sex ratios did not vary significantly by device ($0.25 < p < 0.50$). Pregnancy outcomes did not differ by contraceptive previously used ($0.25 < p < 0.50$, Table IV). Apart from the two premature births all children were reported as healthy or normal.

Comment

Women who had stopped use of any of four new contraceptives to become pregnant conceived at rates (77 to 84 per 100) within the sampling error of 85 per 100 for the 1-year rate demographers regard as typical.[14] Two years after devices were removed, pregnancy rates ranged from 87 to 92 per 100 by method formerly used. At neither 1 nor 2 years did pregnancy rates differ significantly by former method.

Fecundability, the probability of conception in the first month, was depressed among women who used the levonorgestrel IUD, especially among those who experienced no menstrual bleeding in the 90 days preceding device removal. Nevertheless, women who had used this IUD had the same normal cumulative pregnancy rates at 1 and 2 years as did women whose contraception was with the other study methods. Users of the two types of levonorgestrel-releasing implant experienced normal fecundability and pregnancy rates. The Norplant and Norplant II systems thus differ from progestin-only injectables in which women must wait 1 to 3 months or more for the injected drug to disappear before effectively stopping contraception and for which an initial delay of conception has been recorded.[2-4]

Pregnancy rates were significantly associated with age at removal and family planning intentions at study admission. Among women <30 years old, the 2-year probability of pregnancy, 93 per 100, was 13 per 100 higher than that of women ≥30 years old. Similar observations concerning age in previous IUD studies in an implant study and in the general population have been reported.[15-21]

Duration of use, dichotomized at 3 years, had no significant impact on cumulative pregnancy rates for any of the methods. With age and prior family planning intention held constant, longer duration of use (≥37 months) seemed to be associated with moderately increased pregnancy rates but not significantly so. Diaz et al.[19] suggest duration has no effect on planned pregnancy after 5 years of Norplant use. Absence of duration effects on recovery of fertility has been suggested by several authors for former IUD users.[15-19, 22]

Initial family planning intention to have another child was associated with increased pregnancy rates, when duration of use and age were held constant. The

data suggest that women whose family planning intentions had changed may have remained ambivalent about the planned pregnancy and this ambivalence may have affected the frequency of their exposure to unprotected intercourse.

The current study also suggests that women who experienced both pelvic inflammatory disease and a subsequent pregnancy before IUD use have no greater difficulty conceiving than do other women who had used IUDs or Norplant capsules or rods.

The proportions of births, spontaneous abortions, and ectopic pregnancies in this study agree with those found in large studies of former users of contraception.[23, 24] Contemporary estimates of ectopic pregnancy rates have been >1% of recognized pregnancies for several years in the United States and northern Europe, well above the 0.4% observed in this study.

The findings of a significant effect of initial family planning intention on subsequent pregnancy rates suggests potential difficulties or pitfalls in studies that ascertain recovery of fertility among women who stop using contraception because of side effects. Among such women pregnancy rates may be affected by ambivalence with respect to pregnancy.

Women in this study represent a self-selected group who stopped contraception to become pregnant. Thus the generality of these findings may be limited. Case-control studies of infertility and contraception are affected by similar limitations, derived from the self-selection of subjects. In studies of tubal infertility after IUD use cases have been women who sought medical evaluation of infertility. Cases have differed significantly from controls in income[5] and geographic location,[6] as well as in smoking and sexual behavior. Markedly incomplete coverage of cases and controls has also characterized these case-control studies of relative risks of infertility after contraception.

In this study of women desiring pregnancy after contraception, the 1-year life-table rate of conception was 82 per 100. The complement of that rate, 18 per 100, does not materially differ from the 17 per 100 rate of secondary subfertility estimated by Greenhall and Vessey.[25] We conclude that neither the recovery of fertility nor pregnancy outcomes are adversely affected by any of these four new methods of contraception.

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Progression of diabetic retinopathy in pregnancy: Association with hypertension in pregnancy

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OBJECTIVE: To test the hypothesis that women with insulin-dependent diabetes and chronic or pregnancy-induced hypertensive disorders are at increased risk for developing retinopathic complications during pregnancy.

STUDY DESIGN: One hundred fifty-four women with insulin-dependent diabetes were prospectively followed in an intensive program of diabetes in pregnancy. Ophthalmologic evaluations were obtained through pregnancy and at 6 to 12 weeks post partum, and findings were graded by a standard scale. Association of retinopathic progression with risk factors was tested with χ^2 and multiple logistic regression analysis.

RESULTS: Fifty-one women had progression of retinopathy during pregnancy; postpartum regression was observed in 13 women. Changes in glycemic control early in pregnancy, chronic hypertension, and pregnancy-induced hypertension were significantly associated with progression of retinopathy.

CONCLUSION: Women with insulin-dependent diabetes who have hypertensive disorders in pregnancy are at increased risk for progression of retinopathy. (Am J Obstet Gynecol 1992;166:1214-8.)

Key words: Diabetes, retinopathy, pregnancy, pregnancy-induced hypertension

The effect of pregnancy on the natural course of diabetic retinopathy is a matter of controversy.[1] Although some patients with insulin-dependent diabetes experience rapid worsening of retinopathy during pregnancy, others do not. Moreover, retinal changes observed during pregnancy frequently improve post partum.[1]

Systemic hypertension is also associated with retinal changes, and advanced diabetic retinopathy may be associated with lesions similar to those caused by atherosclerosis and arterial hypertension. We therefore hypothesized that women with insulin-dependent diabetes with chronic or pregnancy-induced hypertensive disorders are at increased risk of developing retinopathic complications during pregnancy.

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Material and methods

From 1978, women with type I insulin-dependent diabetes with a history of diabetic ketoacidosis have been enrolled in our continuing program of diabetes in pregnancy at the University of Cincinnati.

Complete medical and obstetric histories were obtained on enrollment. Pregnancy dating was based on menstrual dates and confirmed by physical and ultrasonographic examination. Subjects were examined every 1 to 2 weeks, at which time blood glucose level, body weight, blood pressure, results of urinalysis, and insulin dose were recorded. Glycemic control was maintained with split-mixed-dose regimens of short- and intermediate-acting insulin preparations, along with dietary regulation. The goals of glycemic control were a fasting blood glucose concentration of <100 mg/dl and a 90-minute postprandial glucose concentration <140 mg/dl. Patients were hospitalized when necessary to maintain these goals. In addition, after 1982 patients routinely performed and recorded self-monitoring of blood glucose with reflectance meters. Maternal blood glycohemoglobin A_1 concentrations were measured at entry and every 4 weeks thereafter by Isolab column chromatography (interassay coefficient of variation 7.2%, normal range 5.5% to 8.5%).[2]

Patients underwent ophthalmologic evaluations by one of two ophthalmologists once each trimester and 6 to 12 weeks post partum. Direct and indirect ophthalmoscopy, documented with color photography, was performed at the time of evaluation. Retinopathy was

Table I. Classification of diabetic retinopathy

Grade	*Criteria*
0	Normal fundi
Background retinopathy	
1	Mild background retinopathy: microaneurysms only (one or more)
2	Moderate background retinopathy: microaneurysms, hemorrhages, and soft and hard exudates
3	Extensive background retinopathy: microaneurysms, soft or hard exudates, intraretinal microvascular abnormalities, and venous bleeding
Proliferative retinopathy	
4	Mild proliferative retinopathy: optic disc neovascularization less than standard picture 10c of Diabetic Retinopathy Study[3] or retinal neovascularization less than standard picture 7
5	Moderate proliferative retinopathy: optic disc or retinal neovascularization equal to or more than standard pictures 10c and 7
6	Extensive proliferative retinopathy: optic disc or retinal neovascularization with fibrous proliferation, retinal elevation, or preretinal or vitreous hemorrhage

graded according to funduscopic findings as shown in Table I. The following definitions were used in this study: *progression of retinopathy,* development of retinopathy de novo or upgrading of retinopathy from first or second trimester to later trimesters or post partum or changes in women with grade 6 retinopathy requiring additional photocoagulation; *pregnancy-induced hypertension,* more than one occurrence, 24 hours apart, after 20 weeks' gestation of a mean arterial pressure ≥105 mm Hg or an increase ≥20 mm Hg from baseline; *chronic hypertension,* presence of mean arterial pressure ≥105 mm Hg before 20 weeks' gestation or chronic hypertension diagnosed before pregnancy with or without history of use of antihypertensive medications before pregnancy; *chronic hypertension with superimposed pregnancy-induced hypertension,* an increase of ≥20 mm Hg in mean arterial pressure from baseline after the first 20 weeks of gestation in a patient previously defined as a chronic hypertensive. The current analysis includes all women who had at least two ophthalmologic evaluations during pregnancy, including one in the first or second trimester and one in the third trimester or post partum. In the absence of a third-trimester evaluation (because of either early delivery or noncompliance), a postpartum evaluation showing progression of retinopathy was considered indicative of progression occurring during late pregnancy. Changes in a patient were defined whether present in one or both eyes.

Data were analyzed with SAS (Statistical Analysis Systems, Cary, N.C.). χ^2 was used for categorical variables and the Student t test for continuous variables. The logistic regression model included variables found to be significant on univariate analysis. A p value <0.05 was considered significant.

Results

One hundred fifty-four women with insulin-dependent diabetes had at least two ophthalmologic evaluations during pregnancy (49 had four evaluations, 91 had three, and 14 had two); 51 women (33%) had progression of retinopathy during pregnancy. Retinopathy progressed in 37 women by one grade, in nine by two grades, and in five by three grades or more. Spontaneous improvement from grade 2 in the first trimester to grade 1 in the second was observed in two women. Factors associated with progression of retinopathy during pregnancy are presented in Table II. Women with advanced White class were more likely to have progression than women of White class B or C ($p = 0.01$). Progression of retinopathy was significantly associated with duration of diabetes ($p = 0.04$) and with first-trimester retinal grade ($p = 0.01$). First-trimester mean glycohemoglobin and the magnitude of decrease in mean glycohemoglobin from first to second trimester were also significantly positively associated with retinal changes ($p = 0.01$).

Twenty-two women had development of pregnancy-induced hypertension and three of the 18 women with chronic hypertension had development of superimposed pregnancy-induced hypertension. The associations of retinal changes with hypertensive disorders are outlined in Table III. Women with pregnancy-induced hypertension were significantly more likely than normotensive women to have progression of retinopathy. Women with chronic hypertension (with or without superimposed pregnancy-induced hypertension) were at high risk for progression of retinopathy during pregnancy.

Seven women (three without pregnancy-induced hypertension, two with pregnancy-induced hypertension, two with superimposed pregnancy-induced hypertension) with preexisting background retinopathy had proliferative changes during the course of pregnancy that necessitated photocoagulation therapy. Thirteen of the 51 women who had retinopathic progression during pregnancy experienced postpartum regression of the changes; the distribution of these 13 women was

Table II. Factors associated with progression of retinopathy during pregnancy

	No progression (n = 103)	*Progression (n = 51)*	*Significance (p)*
White class			
B-C	60 (77%)	18 (23%)	
D	33 (59%)	23 (41%)	0.01*
R-F-RF	10 (50%)	10 (50%)	
Age (yr)	25.5 ± 4.6	25.6 ± 4.6	NS
Age at diagnosis of diabetes (yr)	14.7 ± 7.5	12.5 ± 5.6	0.04
Duration of diabetes (yr)	10.9 ± 6.8	13.1 ± 5.8	0.04
First-trimester retinal grade			
0 (no retinopathy)	60 (77%)	18 (23%)	
1-3 (background retinopathy)	40 (59%)	28 (41%)	0.01†
4-6 (proliferative retinopathy)	3 (37%)	5 (63%)	
Mean glycohemoglobin (%)			
First trimester	9.3 ± 1.6	10.3 ± 2.0	0.1
Second trimester	7.7 ± 1.2	7.9 ± 1.4	NS
Third trimester	7.7 ± 1.2	7.7 ± 1.1	NS
Mean glycohemoglobin decrease from first to second trimester	1.6 ± 1.2	2.4 ± 1.6	0.01

Data presented as number and percent or mean ± SD. *NS*, Not significant.
*Association of progression with White classification (χ^2).
†Association of progression with first-trimester retinal grade (χ^2).

Table III. Progression of diabetic retinopathy in pregnancy: Association with hypertensive disorders

	No.	*No progression (n = 103)*	*Progression (n = 51)*	*Significance (p)*
No hypertensive disorder	114	85 (75%)	29 (25%)	
				0.02
Pregnancy-induced hypertension	22	11 (50%)	11 (50%)	
Chronic hypertension (with or without superimposed pregnancy-induced hypertension)	18	7 (39%)	11 (61%)	0.001
All hypertension (pregnancy-induced, chronic, superimposed pregnancy-induced)	40	18 (45%)	22 (55%)	0.0001

as follows: 7 of the 29 normotensive (24%), 3 of the 11 with pregnancy-induced hypertension (27%), and 3 of the 11 with chronic hypertension (27%). None of the women who had proliferative changes during pregnancy had spontaneous regression of the lesions.

Multiple logistic regression analysis was performed to determine which of the univariately significant variables were independently associated with progression of retinopathy. Results indicated that the magnitude of decrease in glycohemoglobin from first to second trimester, pregnancy-induced hypertension, and chronic hypertension were all independently associated with retinopathic progression ($p = 0.004$, $p = 0.01$, $p = 0.02$, respectively). The correlation between first-trimester glycohemoglobin and glycohemoglobin decrease from first to second trimester was 0.71 ($p = 0.0001$); higher first-trimester glycohemoglobin concentrations were associated with greater decreases in glycohemoglobin from first to second trimester. The interaction between these two variables with regard to progression of retinopathy was nonsignificant ($p = 0.7$).

Odds ratios for retinopathic progression were calculated with regard to chronic hypertension, pregnancy-induced hypertension, and a decrease of >2% in glycohemoglobin in women with first-trimester values >8.5%. Odds ratios (with 95% confidence limits) were 2.1 (1.7 to 3.6), 1.8 (1.1 to 3.1), and 1.4 (0.9 to 2.2), respectively.

Comment

Several reports indicate that chronic hypertension may be associated with retinopathy in diabetic patients.[4, 5] However, the association of pregnancy-induced hypertension in an otherwise normotensive pregnant women with insulin-dependent diabetes and progression of retinopathy has not been previously elucidated. Although some authors have found no such association,[6, 7] others have noted a coincidence of pregnancy-induced hypertension and proliferative retinopathy.[8, 9] Most reports do not include an analysis of such possible associations. From our data we conclude that the presence of any hypertensive disorder of pregnancy is associated with progression of diabetic retinopathy.

This conclusion holds true both for previously normotensive women who have development of pregnancy-induced hypertension and, in general, for women with any kind of hypertensive disorder in pregnancy.

There is evidence to suggest that the normal regulatory mechanisms controlling microvasculatory flow are impaired in diabetes[10, 11] and that this impairment parallels the progression of vascular disease. Although mechanisms underlying the association of diabetic retinopathy with hypertension are unclear, it is conceivable that alterations in blood pressure constitute an additional detrimental stress on an already impaired autoregulatory mechanism of blood flow control. Indeed, even intermittent alterations in blood pressure may produce significant blood flow changes in retina vessels[12] and aggravate retinal microangiopathy.[13] Thus the transient hypertension of pregnancy may have a detrimental effect on the already vulnerable diabetic retina. In addition, the mechanisms underlying changes in systemic blood flow and pressure in pregnancy-induced hypertension might also exert a direct effect on retinal microvasculature.

Our findings support previous observations that in pregnant and nonpregnant women with insulin-dependent diabetes the duration of diabetes and the severity of existing retinopathy are major determinants of the progression of retinal disease.[12] Several authors have reported an association between worsening retinopathy and the induction of intensive insulin therapy in nonpregnant subjects with insulin-dependent diabetes.[14-16] Phelps et al.[17] found such an association in women with insulin-dependent diabetes enrolled in an intensive treatment program during pregnancy. In our study a strong association was found between the occurrence of retinal changes and the change in glycohemoglobin from first to second trimester, reflecting the magnitude of change in glycemic control instituted early in pregnancy. However, we also found an association between retinal change and mean first-trimester glycohemoglobin, reflecting glycemic control during the initial stage of pregnancy. The women with higher first-trimester glycohemoglobin values are also those in whom the greatest reduction in glycohemoglobin is to be expected once they enter an intensive program of diabetes in pregnancy. Indeed, the correlation between these two variables was found to be highly significant, whereas the interaction between them was nonsignificant, which would imply that they have no independently additive effect on progression of retinopathy. Thus progression of retinopathy during pregnancy may be associated with either of the following factors: institution of rapid glycemic control in the first trimester or, alternatively, poor glycemic control early in pregnancy. We believe the latter hypothesis to be true and support the opinion of Phelps et al.[17] advocating the pursuit of good glycemic control in diabetic women as early in pregnancy as possible, because the association of poor glycemic control with adverse pregnancy outcome is now widely recognized.[18] Moreover, it has been shown that the accelerated progression of diabetic retinopathy observed in patients receiving intensive insulin therapy is not sustained over time and that these patients tend to have less overall deterioration over the course of 2 years.[19] Preconceptional institution of intensive insulin therapy has been associated with improved pregnancy outcome[20-22]; it remains to be seen whether patients may benefit from such an approach with regard to progression of retinopathy and whether preconceptional normalization of glycohemoglobin is associated with a lower risk for progression of retinopathy during pregnancy.

It is clear that at least in some women with insulin-dependent diabetes who experience progression of background retinopathy during pregnancy postpartum regression of these changes may be expected.[7, 23] How long it may take for such regression to be evident is unclear, and we cannot exclude the possibility that some of the women in our study experienced improvement after the 6- to 12 week-postpartum examination. Because pregnancy-induced hypertension is a transient phenomenon related to pregnancy, it might be expected that the rate of postpartum regression of retinopathic changes would be higher in women with insulin-dependent diabetes who have pregnancy-induced hypertension than in chronically hypertensive or normotensive women. However, we found that the rate of postpartum retinopathic regression was similar among women with or without pregnancy-induced hypertension. This observation supports the contention that even transitory changes in blood pressure may exert permanent retinopathic effects.

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Prenatal ultrasonographic findings associated with wide clefting of the upper two thirds of the fetal sternum

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Complete or partial absence or clefting of the fetal sternum is uncommon. A teenage primiparous patient at 34 weeks' gestation with severe preeclampsia was delivered of an infant with wide clefting of the upper two thirds of the sternum. The infant had no respiratory or cardiac symptoms. There were no other congenital abnormalities. Corrective neonatal surgery was performed at 1 month of age. Retrospective analysis of the ultrasonographic examination performed at 34 weeks revealed evidence of this fetal sternal defect. (AM J OBSTET GYNECOL 1992;166:1219-21.)

Key words: Partial cleft of fetal sternum, ultrasonography

Partial absence of the fetal sternum is a rare occurrence. Three instances of complete absence of the sternum have been reported since 1979.[1] More commonly, clefting, perforation, or indentation of the sternum has been observed.

We present a case of the prenatal ultrasonographic findings associated with wide clefting of the superior two thirds of the fetal sternum.

Case report

A 14-year-old, gravida 2, para 0, Hispanic female patient presented for prenatal care at 30 weeks' gestation. Ultrasonography at that time revealed a singleton fetus with what appeared as normal fetal anatomy and growth parameters that were appropriate for gestational age.

She was hospitalized at 34 weeks 1 day of gestation with preeclampsia. She was asymptomatic. Blood pressure ranged between 140 and 170/100 and 110 mm Hg and she had proteinuria (2+). Physical examination was remarkable for a fundal height of 27 cm, 3+ deep tendon reflexes, and trace pedal edema. Fetal heart rate monitoring revealed a reactive nonstress test.

Intravenous magnesium sulfate was continued at a rate of 2 gm/hr after an intravenous loading dose of 4 gm. Subsequent blood pressure measurements taken in the left lateral recumbent position were between 130 and 140/80 and 100 mm Hg. Ultrasonography on admission revealed a vertex-presenting infant with reduced amniotic fluid volume. The estimated fetal weight was 1895 gm, which is the 20th percentile for 34 weeks' gestation.

Laboratory values of significance included a uric acid level of 7.1 mg/dl. (The results of a 24-hour urine collection were available after delivery and revealed >13 mg of protein and a creatinine clearance of 132 ml/min.) On the third hospital day, fetal monitoring revealed a reactive nonstress test with decreased beat-to-beat variability. Blood pressure was 150/100 mm Hg. A subsequent fetal heart rate tracing showed fetal distress with a repetitive late deceleration pattern. The patient underwent a primary low-transverse cesarean section for severe preeclampsia and fetal distress.

A 1625 gm female infant with Apgar scores of 4 and 9 at 1 and 5 minutes, respectively, with thick meconium, was delivered. There was no meconium below the vocal cords. Physical examination of the infant in the delivery room revealed complete absence of the manubrium sterni and wide clefting of the upper two thirds of the body of the sternum (Fig. 1, *A*). The sternal abnormality was U-shaped with the apex at the level of the fifth rib and measured 4 cm in width. The lower segment of the sternum, as well as the xiphoid, was intact and together they measured 2 cm in length. The medial aspects of the laterally displaced clavicles and the anterior aspects of the ribs were palpable at the margins of the defect. Cardiac activity was easily observed through the intact skin. There were no other abnormal findings.

The infant did not manifest any respiratory symptoms. An electrocardiogram and an echocardiogram were normal. Chest x-ray film showed lateral displacement of the clavicles with normal configuration of the ossified portions of the 12 ribs (Fig. 1, *B*). A computerized tomographic scan of the thorax revealed absence of the upper two thirds of the sternum. Retrospective analysis of the ultrasonographic examination per-

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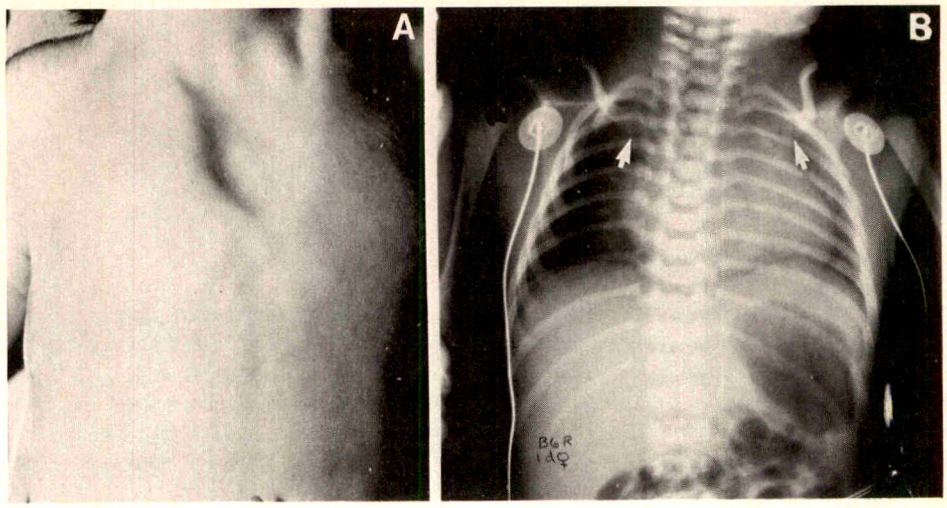


Fig. 1. A, Photograph of anterior neonatal chest wall. Note wide midline sternal cleft. **B**, Anteroposterior radiographic view of neonatal chest. Note lateral displacement of medial aspects of clavicles *(arrows)*.

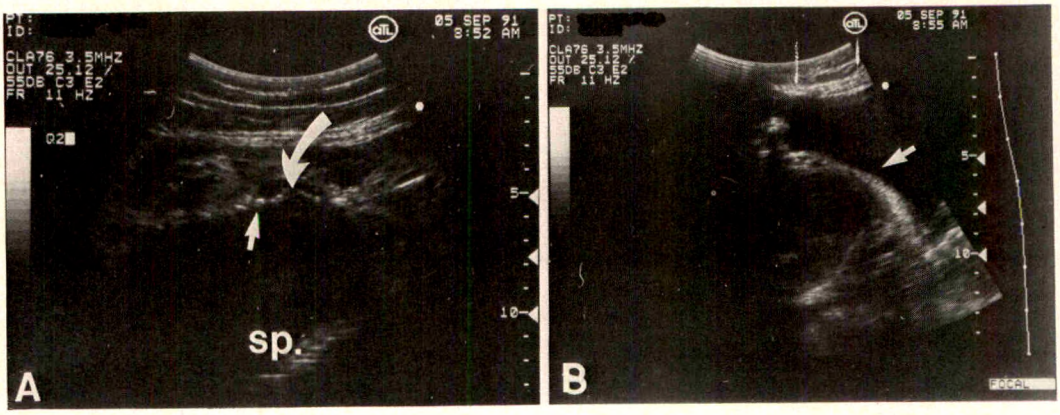


Fig. 2. A, Longitudinal ultrasonographic view of fetal chest in spine *(sp.)*–down position at 34 weeks. Calcification of lower aspect of the sternum *(small arrow)*. Note clefting of upper sternum *(curved arrow)*. **B**, Transverse ultrasonographic view of fetal chest at level of four-chamber view of heart. Note absent midline sternal calcification *(arrow)*.

formed at 34 weeks suggested evidence of the fetal sternal defect (Fig. 2). Because of potential injury to the unprotected neonatal heart, corrective surgery of the anterior chest wall was performed on the infant at 4 weeks of age. The diagnosis of an absent manubrium sterni and wide superior sternal clefting was confirmed at operation, and the infant's postoperative course was unremarkable.

Comment

The sternum originates from the lateral plate mesoderm that will also form the pectoralis muscle. The earliest indication of the sternum is a pair of mesenchymal bands that can be identified in the human embryo at 6 weeks.[1] These bands lie ventrolaterally in the body wall and initially have no connection with the ribs or with each other. After attachment of the ribs to the sternal bars, the paired bands unite progressively in a craniocaudal direction. At 9 weeks the union of the cartilaginous bands is complete. The cranial end of the developing sternum incorporates two separate suprasternal cartilages with which the clavicles articulate. These cartilages join the manubrium of the sternum and subsequently lose their identity. The ossification centers of the sternum normally appear later in the midline, at approximately 20 to 24 weeks' gestation.

Congenital absence of the sternum is usually seen in association with other fetal anomalies such as ectopia cordis or pentalogy of Cantrell (midline supraumbilical abdominal defect, defect of the lower sternum, defi-

ciency of the diaphragmatic pericardium, deficiency of the anterior diaphragm, and intracardiac anomalies). Various degrees of isolated sternal abnormalities have been described. The most common defect is failure of the sternal bars to fuse in the midline.[2] Nutritional deficiencies have been associated with skeletal deformities. Hague and Zaidi[2] described two cases of complete absence of the sternum in infants born to nomadic Saudi Arabian mothers. Both women lived in a region where essential vitamins were often absent from the diet. These authors suggested that chronic nutritional deficiencies, especially of riboflavin, may be associated with this skeletal anomaly. Our 14-year-old patient, socioeconomically disadvantaged female showed no signs of malnutrition.

Surgical repair of superior sternal clefting is usually performed as early as the infant's condition will permit. As the infant grows older, the cartilage becomes less malleable and the thoracic contents move anteriorly into the defect. In V-shaped defects the edges of the sternum can often be approximated by suture closure with relaxing osteotomy of the edges if needed. U-shaped defects often require transection of the limbs of the U at the juncture with the normal sternum, followed by suture approximation. Wire or synthetic mesh prosthesis or a bone graft may be required if the defect is very large or difficult to close.

To the best of our knowledge, this case is the first description of prenatal ultrasonographic findings associated with isolated clefting of the fetal sternum.

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The presence of platelet-activating factor binding sites in human myometrium and their role in uterine contraction

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It has recently been suggested that platelet-activating factor is involved in a number of processes associated with reproductive biology. We have demonstrated a critical role for this autacoid in the initiation and maintenance of parturition both in animals and man. In the present investigation, it has been demonstrated that platelet-activating factor affects contraction in strips of human myometrium at concentrations as low as 10^{-10} mol/L. A platelet-activating factor receptor has been identified and characterized in human myometrium. Platelet-activating factor acts to cause an increase in intracellular Ca^{2+} concentration of isolated myometrial smooth muscle cells in culture and an increase in the phosphorylation of the 20 kd light chain of myosin in a concentration-dependent fashion. The presence of platelet-activating factor receptors in human myometrium, the observed increase in Ca^{+2} concentration and phosphorylation of myosin light chain in the presence of platelet-activating factor provide further support for the importance of this autacoid in the initation of parturition. (AM J OBSTET GYNECOL 1992;166:1222-8.)

Key words: Platelet-activating factor, myometrium, platelet-activating factor receptors, calcium, myosin light chain phosphorylation

Prostaglandins have been associated with reproductive biology since their discovery in the early thirties. More recently, a second autacoid, namely platelet-activating factor (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF) has also been implicated in many of these processes, including ovulation,[1] sperm motility,[2] embryo implantation,[3] and the initiation of parturition and fetal lung maturation.[4, 5] In previous studies it was reported that PAF could be isolated and identified in amniotic fluid obtained from women at term and in active labor.[6] PAF was not present in amniotic fluid obtained from women at term who were not in labor. These observations were confirmed by Nishihira et al.[7] PAF affects contraction of rat,[7] guinea pig,[8] and human myometrium.[9] It has been suggested that PAF in amniotic fluid is, at least in part, of fetal lung origin.[10] PAF metabolism in the maternal compartment may also be important throughout gestation. The enzyme PAF acetylhydrolase catalyzes the inactivation of PAF to form lysoPAF. In the rabbit, the activity is decreased to 10% of the nonpregnant level in the maternal plasma during the last half of pregnancy.[11] After delivery, the PAF acetylhydrolase activity returns to prepregnancy levels within 24 hours. A decrease in PAF acetylhydrolase activity has also been demonstrated in the maternal plasma of the human.[12] It has been suggested that the combination of increased PAF synthesis in the fetal compartment, in combination with the decreased capacity of the maternal compartment to inactivate the autacoid, may promote delivery of PAF along with other autacoids, such as prostaglandins, to the myometrium, where they act to stimulate myometrial contractions.[13] PAF binding in endometrium has been characterized,[14] and the presence of a PAF receptor in rabbit myometrium suggested.

In the current investigation we report the presence of PAF receptors in human myometrium. We have also investigated the molecular basis of PAF action in human myometrial smooth muscle cells in culture by quantifying changes in the intracellular Ca^{2+} concentration and the extent of myosin light chain phosphorylation in these cells.

Material and methods

Human myometrial smooth muscle cells in culture. Human uterine specimens were obtained from premenopausal women at the time of hysterectomy performed for reasons other than endometrial or myometrial disease. Myometrial smooth muscle cells were isolated and maintained in primary and first-passage monolayer cultures as previously described[15] in Ham's F12–Dulbecco's minimal essential medium that contained fetal bovine serum (10%) and antibiotic-anti-

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mycotic solution (10,000 U/ml penicillin G, 10,000 μg/ml steptomycin, and 25 μg/ml amphotericin B; 1% by content). Cells were incubated in a serum-free medium that contained human serum albumin (0.4%) for 16 hours before experiments.

Preparation of membrane fraction for PAF binding studies. Fresh human myometria denuded of endometrium were minced into very small pieces (1 to 3 mm^3) and then homogenized with a Polytron device (Kinematica, GmbH, Lucerne, Switzerland) for 3 minutes at 0° C in eight volumes (wt/vol) of prechilled Tris hydrochloride buffer (50 mmol/L, pH 7.6) at a setting of 5. Plasma membrane was prepared with a slight modification of the method previously described.[16, 17] The homogenate was filtered through two layers of cheesecloth, followed by centrifugation at 1000 *g* for 10 minutes at 4° C. The resulting pellet was resuspended in magnesium chloride (5 mmol/L), Tris (10 mmol/L), and ethylenediaminetetraacetic acid (2 mmol/L), pH 7.0, and fractionated on a discontinuous sucrose density gradient between 10%, 30%, 35%, 40%, and 50%. The membrane fragment used banded at the interface between the 35% and 40% sucrose solution. The specific activity of the plasma membrane marker enzyme[18] alkaline phosphatase had the highest specific activity in this fraction. This fraction had the highest PAF binding and was identical to the fraction reported for other tissues.[16, 17] The PAF receptor antagonist L-659,989 was used to further characterize the binding.[19] The membranes were stored at −80° C until use. No significant loss of tritium-labeled PAF specific binding was found during storage for up to 2 weeks. The protein concentration was determined by the method of Lowry et al.[20] with bovine serum albumin used as the standard.

PAF receptor binding assay. The specific binding of tritium-labeled PAF to the membranes of myometrium was assayed in a manner similar to that previously described by Hwang et al.[17] The membranes were suspended in magnesium chloride (10 mmol/L), Tris (10 mmol/L), and bovine serum albumin (0.25%) at pH 7.0. The suspension was incubated with tritium-labeled PAF in a total volume of 1 ml in the presence or absence of various concentrations of nonradioactive PAF (range 0.04 to 2.5 nmol/L) for 2 hours at 0° C. Nonspecific binding was determined by the addition of a 1000-fold excess on nonlabeled PAF. Binding was terminated by rapid vacuum filtration over a filter (HVLP 01300, Millipore Corp., Bedford, Mass.), followed by five 1.5 ml rinses with cold (4° C) buffer. The radiolabeled PAF present on the filter was counted by liquid scintillation spectrometry. The specific binding was computed as the difference between total binding and nonspecific binding. The PAF receptor antagonist studies were carried out with a method similar to that previously described.[19]

Quantification of intracellular Ca^{2+} concentration. The cultured cells were removed from the flask by treatment with Hank's balanced salt solution containing trypsin (0.05%, wt/vol, ethylenediaminetetraacetic acid (0.6 mmol/L), and Hepes (10 mmol/L, pH 7.4) for 5 to 8 minutes. The cells were then pelleted by centrifugation, washed two times, and incubated at 25° C for 15 minutes in culture medium that contained fura-2 acetoxymethyl ester (0.5 μmol/L). Thereafter, the cells were centrifuged, washed, and suspended a concentration of 1×10^6 cells per milliliter in buffer that contained potassium chloride (4.8 mmol/L), sodium chloride (130 mmol/L), magnesium chloride (1 mmol/L), calcium chloride (1.5 mmol/L), sodium phosphate (1 mmol/L), glucose (15 mmol/L), and Hepes (10 mmol/L, pH 7.4), as previously described.[21] Intracellular Ca^{2+} concentration was determined with a Perkin-Elmer 650-10S fluorescence spectrometer (Perkin Elmer, Norwalk, Conn.) by quantification of fluorescence with 340 nm excitation and 510 nm emission wavelengths, as described by Tsien et al.[22] and implemented in our laboratory.[21]

Determination of phosphorylation of light chain of myosin. The phosphorylation of myosin light chain was determined by densitometric analysis of myosin light chain (nonphosphorylated and monophosphorylated) that was visualized on immunoblots, as described.[23] The data are expressed as the percent of the total immunodetectable 20 kd myosin light chain that is phosphorylated.

Contraction of human uterine smooth muscle strips. Uterine longitudinal smooth muscle strips were prepared from the human uterus as described previously.[24] Before the addition to the test solution, individual strips were stretched to optimal length for force development and conditioned by a single contraction in physiologic saline solution that contained potassium chloride (65 mmol/L) substituted isotonically for sodium chloride. PAF was added to the incubation media in 2.5% bovine serum albumin that had been dissolved in normal saline solution and adjusted to pH 7.4. Control incubations contained bovine serum albumin alone.

Chemicals. Tritium-labeled PAF (1-O-[hexadecyl-1′-2′-^{3}H(N)]-2-acetyl-*sn*-glycero-3-phosphorylcholine), was purchased from New England Nuclear, Boston, with a specific activity of 10 Ci/mmol. Unlabeled C16-PAF was obtained from Avanti, Pelham, Ala., and from Sigma Chemical Co., St. Louis. L-659,989 was kindly supplied by Dr. J.C. Chabala of Merck Sharp & Dohme, Rahway, N.J.

Statistical analysis. The values given are the means ± SEMs.

Results

PAF receptor. The specific binding of tritium-labeled PAF to human myometrial plasma membrane was com-

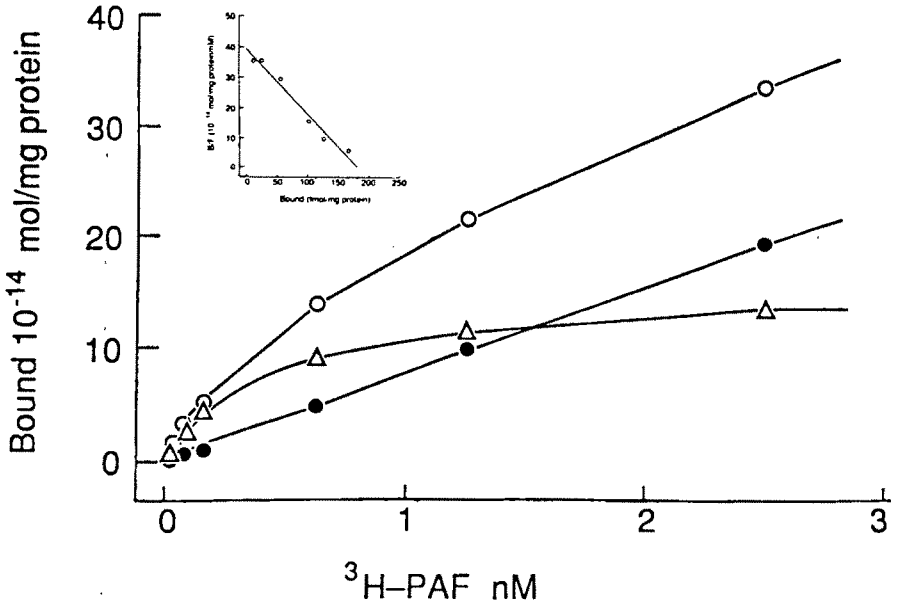


Fig. 1. Binding of tritium-labeled PAF to plasma membrane fraction prepared from human myometrium (○—○, total binding; ●—●, nonspecific binding; △—△, specific binding). *Inset*, Scatchard analysis of PAF receptor binding. Dissociation constant and maximal binding sites were 0.38 and 155.8 fmol/mg protein, respectively.

puted by the subtraction of the nonspecific binding from the total binding. The binding was determined with a 1000-fold excess of nonradiolabeled PAF (Fig. 1). Scatchard analysis of the PAF receptor for the human myometrium membranes is shown in the insert. This analysis is indicative of a single class of receptor sites for PAF in the membranes of myometrium. PAF receptor maximal binding sites of the human myometrial membranes was computed and found to be approximately 94.3 ± 25.2 fmol/mg protein. The mean equilibrium dissociation constant of PAF receptor of this sample of human myometrial membranes was 0.27 ± 0.05 nmol/L.

We also examined the effect of PAF receptor antagonist L-659,989 on the binding of PAF to the membrane fraction prepared from human myometrium. The membranes were incubated as previously described, with the exception that the PAF antagonist L-659,989 (6 nmol/L) was included in the experimental flasks.[19] A 50% inhibition of PAF binding was observed in myometrial membranes incubated in the presence of L-659,989. The inhibition was similar to that observed in other tissues.[19]

Effect of PAF on uterine contractility. Longitudinal uterine muscle strips from nonpregnant women were treated with PAF at a concentration of 2×10^{-10} mol/L. PAF acted to increase the active force of spontaneous contraction from 2.5 to 4.5 gm, and baseline tone increased from 1 to 3 gm for >20 minutes or until PAF was washed from the bath and replaced with salt solution (data not shown). Rhythmic contractions were superimposed on this elevation in baseline tone. The force of spontaneous contractions and the contractile force in response to PAF was determined by quantification of the area of active force during the 10 minute period just before and the 10-minute period after the addition of PAF. PAF induced a threefold increase in contractile activity. Previously, Tetta et al.[9] also reported a myometrial contractile response in human myometrial muscle strips of the lower uterine segment that were treated with PAF.

Effect of PAF on intracellular Ca^{2+} concentration and myosin light chain phosphorylation in myometrial cells in culture. PAF (10^{-14} to 10^{-10} mol/L) stimulated an increase in intracellular Ca^{2+} concentration (Fig. 2). PAF (10^{-10} mol/l) stimulated a threefold increase in intracellular Ca^{2+} concentration (from 112 to 301 nmol/L). The increase was transient with maximal values observed from 12 to 15 seconds, returning to basal levels by 60 seconds (Fig. 2, inset). Treatment of human myometrial smooth muscle cells with PAF resulted in an increse in phosphorylation of myosin light chain. In cells treated for 15 seconds with PAF at a concentration of 10^{-10} mol/L, myosin light chain phosphorylation increased from 3% (control) to 40% (Fig. 3).

Comment

Numerous investigations have been reported concerning the role of a variety of agents that effect the initiation and maintenance of parturition in various species, including the human. The role of various ei-

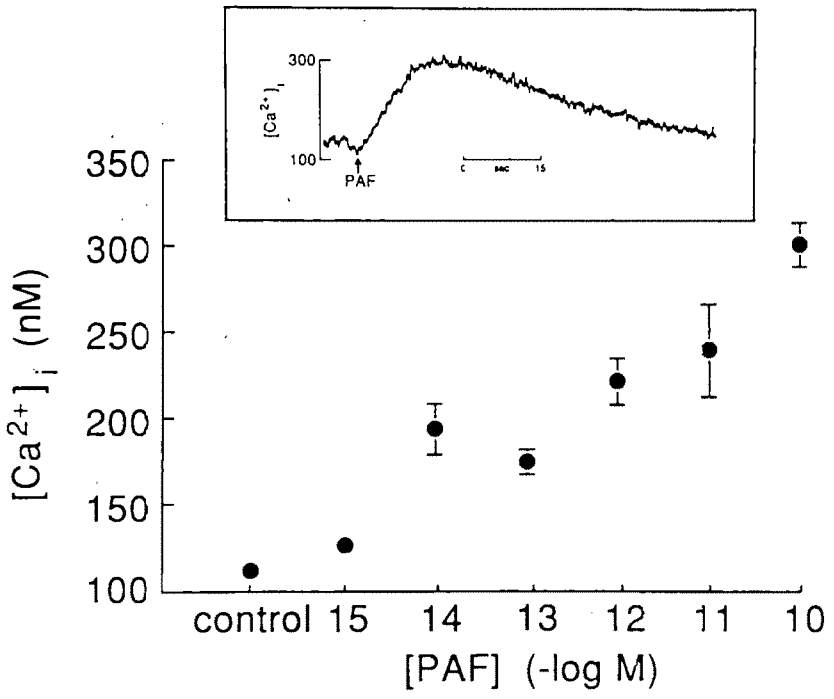


Fig. 2. Effect of PAF in intracellular Ca^{2+} in human myometrial smooth muscle cells. Data are expressed as mean ± SEM for 3 to 10 samples. *Inset*, Recording of fluorescence (340 nm) of fura-2-containing myometrial cells treated with PAF (10^{-10} mol/L). Data are represented as mean ± SEM.

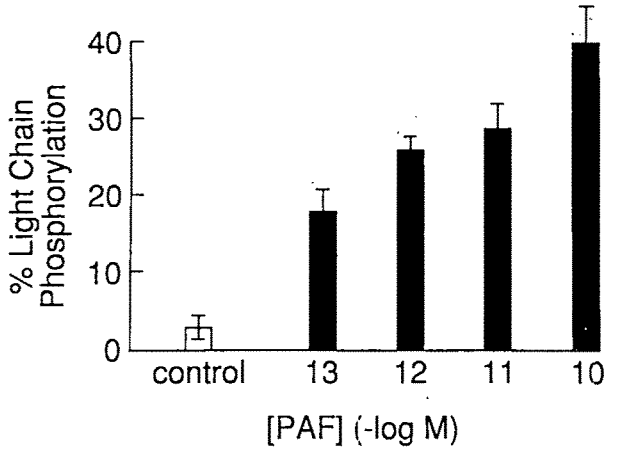


Fig. 3. Effect of PAF on myosin light chain phosphorylation in myometrial smooth muscle cells. Cells were treated with various concentrations of PAF. After 15 seconds, cellular protein was precipitated with trichloraacetic acid (10% wt/vol) and processed for immunoblot analysis after urea-glycerol gel electrophoresis, as described in Material and methods. Data are represented as mean ± SEM of extent (percent) of immunoreactive myosin light chain that is phosphorylated in three to four samples.

cosanoids in the events of parturition has been a major focus.[25, 26] Several years ago it was suggested that PAF may also play a role in this process, and the presence of PAF in the amniotic fluid of women at term and in labor was demonstrated.[6] It was suggested that the tissue origin of the PAF in amniotic fluid was the fetal lung[10] and kidney,[6] not the decidua.[27] The synthesis of PAF and the presence of PAF precursors were demonstrated in fetal membranes, but these tissues have limited capacity for PAF secretion.[28] The role of the fetal lung is implied by the fact that significant amounts of the PAF present in the amniotic fluid are associated with the surfactant-containing lamellar body fraction,[6] and an increase in PAF occurs in the rabbit fetal lung

during development.[10, 29] A direct role for PAF in surfactant biosynthesis was also suggested.[30, 31] PAF will also stimulate prostaglandin E_2 production in amnion tissue and cells.[32, 33] At later stages of gestation, when the fetal tissues have an increased capacity to synthesize PAF, there is a decreased capacity in the maternal plasma to inactivate PAF because of the decrease in plasma PAF acetylhydrolase activity.[11, 12] Early in pregnancy the small amounts of PAF synthesized may be prevented from reaching the myometrium by high circulating levels of maternal plasma PAF acetylhydrolase. The abundant blood supply of the decidua makes it a likely site for the inactivation of PAF.[13]

In the reported results we have confirmed the effect of PAF on the contraction of uterine smooth muscle and have demonstrated and characterized the PAF receptor in the plasma membranes of human myometrium. The characteristics of this receptor are similar to those reported for other tissues.[17] Kudolo and Harper[14] have demonstrated the presence of the PAF receptor in various uterine tissues of the rabbit. Although their efforts have focused primarily on the PAF receptor in the endometrium, they have noted that the receptor is present in the rabbit myometrium, albeit at a much lower density. The recent cloning of the PAF receptor from guinea pig lung,[34] a first among the various lipid mediators, provides further support for the concept that PAF exerts its action by a receptor-mediated event. In this study the receptor has been demonstrated and characterized from nonpregnant women. We have demonstrated previously that the administration of the PAF receptor antagonist L-659,989 to pregnant rats causes a prolongation of parturition.[35] We have concluded that L-659,989 also inhibits PAF binding to a membrane fraction prepared from human myometrium. This PAF receptor antagonist has been reported to be a rather specific inhibitor.[19] Thus the combination of these findings would provide further support for the suggestion that PAF is involved in parturition.[3]

We have also demonstrated that PAF will stimulate the contraction of strips of myometrium at relatively low concentrations (threefold at 10^{-10} mol/L). An increase in intracellular Ca^{2+} concentration and myosin light chain phopshorylation has been observed within seconds of the addition of PAF to human myometrial smooth muscle cells in culture. In most studies in which various agents have been used to stimulate uterine contraction, intracellular Ca^{2+} concentration increase, or myosin light chain phosphorylation, the concentration of the agonist used was between 10^{-6} and 10^{-9} mol/L.[36]

As indicated in Figs. 2 and 3, PAF stimulated Ca^{2+} uptake (Fig. 2) and the phosphorylation of myosin light chain (Fig. 3) at concentrations $<1 \times 10^{-10}$ mol/L. Thus it appears that PAF is one of the most potent stimuli of myometrial contraction. PAF is active in other systems at concentrations of 10^{-10} to 10^{-12} mol/L.[37, 38] The dissociation constant for PAF in the myometrial membranes used in this investigation is of the order of 10^{-10} mol/L. PAF acted to cause an increase in intracellular Ca^{2+} concentration in myometrial cells in culture in concentrations as low as 10^{-14} mol/L. Additional mechanisms must be invoked to account for the mechanism of action of PAF. Recently it has been reported that the PAF receptors present in the plasma membrane of various different cell types are heterogeneous.[39] In addition, this group of investigators has demonstrated the presence of the intracellular receptors in certain tissues.[39] The existence of PAF receptors of the intracellular type in human myometrium and their possible role in the contractile processes is presently under investigation.

In summary, we have demonstrated a receptor for PAF in human myometrium and that human myometrial smooth muscle cells in culture respond to PAF with an increase in intracellular Ca^{2-} concentration and myosin light chain phosphorylation. The PAF receptor antagonist L-659,989 blocks the PAF binding to the membrane fraction. On the basis of these findings and previously published observations,[12, 13] it is suggested that PAF may play an important role in conjunction with other autacoids in the initiation and maintenance of parturition.

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Effects of platelet-activating factor on prostaglandin E_2 production by intact fetal membranes

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OBJECTIVE: The hypothesis tested in this study is that platelet-activating factor increases prostaglandin E_2 synthesis from fetal membranes.

STUDY DESIGN: Fetal membrane disks obtained before or after labor were incubated with or without platelet-activating factor for time periods of up to 24 hours. The production of prostaglandin E_2 and its inactive metabolites was determined by specific radioimmunoassays.

RESULTS: Platelet-activating factor (1 to 10 μmol/L) stimulated the production of prostaglandin E_2 and its metabolites by intact fetal membranes and chorion-decidua threefold to fourfold after 24 hours of incubation. Platelet-activating factor had far greater effects on the production of prostaglandin E_2 by intact fetal membranes obtained after the onset of labor, such that prostaglandin E_2 production was increased by tenfold to 100-fold.

CONCLUSION: These results suggest that platelet-activating factor mainly stimulates prostaglandin E_2 production by the chorion-decidua before labor and that it may act in synergism with other stimulatory factors present in the fetal membranes during labor. (Am J Obstet Gynecol 1992;166:1228-31.)

Key words: Platelet-activating factor, fetal membrane, labor, prostaglandin E_2

The control of normal term human labor and delivery has been extensively studied over the last 15 to 20 years, and although the role of prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ has been established unequivocally,[1, 2] relatively little progress has been made beyond this point. The measurement of increased prostaglandin (and prostaglandin metabolite levels) in preterm and term labor,[3, 4] together with the ability of endogenously administered prostaglandins to initiate labor and delivery,[5] has shown that these compounds have a focal role in the process of labor. However, the factors that control intrauterine prostaglandin production remain to be identified, although activation of the immune response and the release of cytokines have been implicated in preterm labor associated with infection.[6-8] The similarities between pathologic preterm labor and the normal, term process remain unclear, although small increases in cytokines have been shown in amniotic fluid obtained from women with term labor,[6, 8] but definitive data linking these stimuli with the initiation of labor are lacking.

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), a bioactive lipid, has been implicated in labor by the finding that PAF levels in amniotic fluid are elevated in both term and preterm labor.[9-11] PAF is a component of fetal urine and may be secreted by the maturing fetal lung[12]; it may also be released by decidual activation associated with intrauterine infection.[13, 14] The synthesis of PAF is integral to the normal immune response to infective agents,[13, 14] so the production of PAF during preterm labor associated with infection is likely to be part of this general reaction rather than a specific initiation of labor. In term labor the synthesis of PAF from specific tissues[12] may be directed to initiate labor. PAF is known to increase prostaglandin production by a number of tissues,[15, 16] so we investigated the effects of PAF on the production of PGE_2 by cultured intact fetal membrane, the amnion, and the chorion-decidua.

Methods and material

Fetal membranes were obtained after delivery either by elective cesarean section or by normal term labor and vaginal delivery. Fetal membrane disks were prepared and cultured as described elsewhere.[17] In brief, the membranes were cultured on the ends of glass cylinders (outside diameter 14 mm) and held in place by silicone rubber rings (J. Walker & Co., Ltd., Woking, Surrey, England). Excess membrane was removed with a scalpel. Silicone rubber washers (J. Walker) were placed on the other end of the cylinder to support the glass cylinders from the lid of a 12-place multiwell plate

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in which holes with a diameter of 24 mm had been drilled. Tissue culture medium (1.5 ml) was then added to each side of the membrane, to produce a two-compartment experimental system. One compartment was in contact with the amnion (fetal side), and the other was in contact with the chorion-decidua (maternal side). The medium used was medium 199 with antibiotics (Gibco, Paisley, Scotland), 10% heat-inactivated horse serum (Gibco), and 1% L-glutamine (Gibco). An intact multiwell lid was placed over the top of the system to maintain sterile culture conditions, and the culture system was incubated at 37° C in 95% air and 5% carbon dioxide. In all the experiments a 24-hour period of incubation was used before any further experimentation to allow the membrane to recover from the trauma of delivery and the laboratory procedures. Cell membrane integrity was tested with diaphorase histochemical staining,[18] and this was used as an assessment of the viability of the cells in the fetal membranes.[17]

PAF (from Sigma Chemical Co., Poole, Dorset, England) at concentrations from 1 pmol/L to 10 μmol/L was added to either the maternal side, the fetal side, or both sides of the membrane, and the incubation was continued for various times from 4 to 24 hours, after which the medium was removed and frozen at −20° C until measurement of PGE_2 and its metabolites by radioimmunoassays.[19] In some experiments the membrane was split before culture, and separated amnion or chorion-decidua was cultured in the same way as intact fetal membrane. Differences in the production of PGE_2 and its metabolites were assessed by two-way analysis of variance. The radioimmunoassay for PGE_2 was an "in-house" technique, with PGE_2 standard and PGE_2 antibody from Sigma Chemical Co. and tritiated PGE_2 and scintillation proximity assay reagent from Amersham International (Aylesbury, Buckinghamshire, England). PGE_2 metabolites were measured with a kit from Amersham International.

Results

In the initial experiments platelet-activating factor was added simultaneously at the same concentration to both sides of the fetal membrane, and total PGE_2 production on both sides of the membrane was determined. PAF stimulated PGE_2 production by cultured amnion maximally at a concentration of 0.1 μmol/L after 24 hours of incubation (Fig. 1). At shorter incubation times, PAF stimulated smaller increases in PGE_2 production (results not shown). Higher concentrations of PAF (1 to 10 μmol/L) were required to stimulate PGE_2 production from intact fetal membrane (Fig. 1) and chorion-decidua (results not shown). In subsequent experiments the intermediate concentration of 5 μmol/L PAF was used to stimulate PGE_2 production from intact fetal membranes or chorion-decidua.

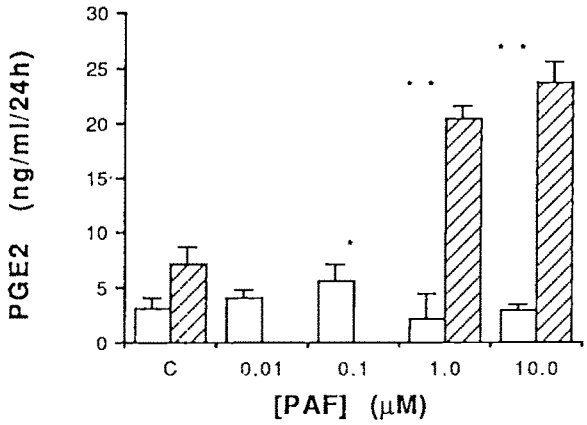


Fig. 1. Production of PGE_2 from amnion *(open box)* or intact fetal membrane *(hatched box)* in response to different concentrations of PAF added simultaneously to both sides of membrane. All data are means ± SEM ($n = 4$) and are representative of three experiments. *One asterisk*, $p < 0.05$ (vs control); *two asterisks*, $p < 0.01$ (vs control).

Further studies on intact fetal membranes showed that PAF added to the amnion side of the intact membrane had little effect on PGE_2 production (Fig. 2, *A*) whereas PAF added to the decidual side of the membrane stimulated PGE_2 production fourfold (Fig. 2, *A*). The addition of PAF to both sides of the fetal membrane increased PGE_2 production on the decidual side, whereas PGE_2 metabolite was increased on both sides of the membrane (Fig. 2, *B*), which suggested that the increase in PGE_2 was unlikely to be due to a decrease in the rate of metabolism. Similar threefold to fivefold increases in PGE_2 and PGE_2 metabolite levels were found when PAF was added to the decidual side of cultured chorion-decidua (results not shown).

Intact fetal membranes obtained after labor produced basal levels of PGE_2 that were higher (Fig. 3) than those in the corresponding tissue obtained before labor (Fig. 2). PAF (5 μmol/L) further increased PGE_2 production more than tenfold, such that PGE_2 production exceeded 100 ng in 24 hours (Fig. 3) but only when it was added to the decidual side of the membrane. PAF added to the amnion side had no effect, and the addition of PAF to both sides of the membrane had less effect than PAF added to the decidual side alone (Fig. 3).

Comment

PAF has been implicated in labor by the finding that increased levels of this substance are present in amniotic fluid during both preterm and term labor,[11] but the sources of this PAF have not been identified. The increased levels of PAF may come from the maturing fetal lung,[12] which may be of importance in term labor, or from activation of the immune response after intra-

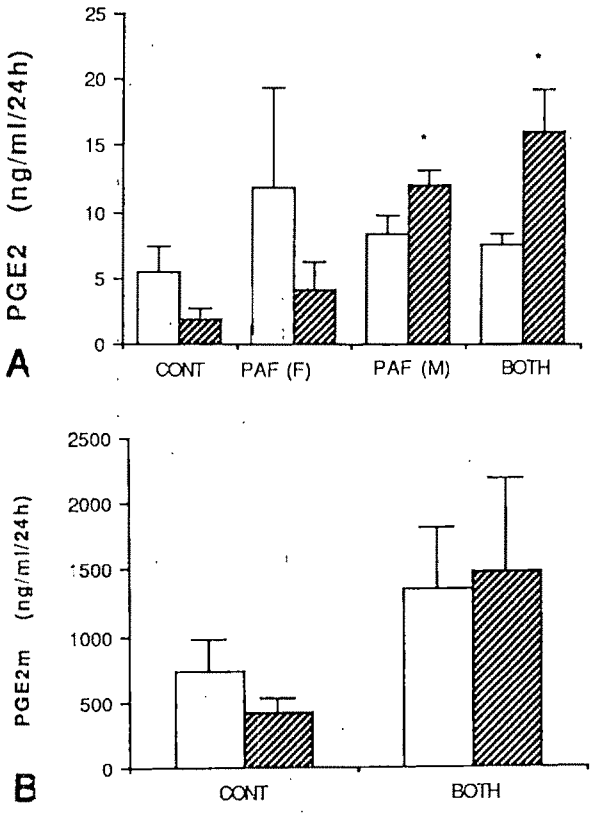


Fig. 2. Production of PGE_2 **(A)** or PGE_2 metabolites **(B)** by intact fetal membranes in presence or absence of PAF (5 μmol/L). PAF was added to fetal *(F)*, maternal *(M)*, or both sides of membrane as shown; and prostaglandin production was measured in medium in contact with amnion *(open box)* or chorion-decidua *(hatched box)*. All data are means ± SEM ($n = 3$) and are typical of three experiments. *One asterisk*, $p < 0.05$ (vs control).

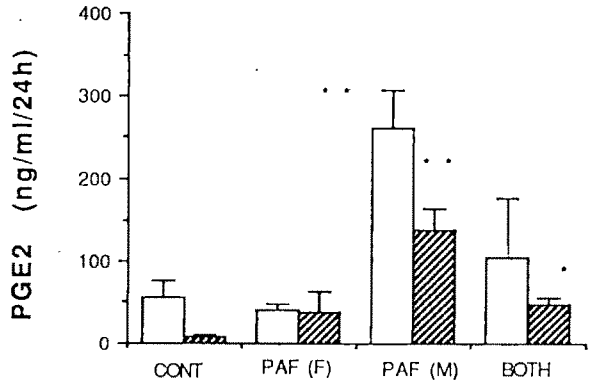


Fig. 3. Production of PGE_2 by intact fetal membranes obtained after labor in presence or absence of PAF (5 μmol/L). PAF was added to fetal *(F)*, maternal *(M)*, or both sides of membrane as shown, and prostaglandin production was measured in medium in contact with amnion *(open box)* or chorion-decidua *(hatched box)*. All data are means ± SEM ($n = 3$) and are typical of three experiments. *One asterisk*, $p < 0.05$ (vs control); *two asterisks*, $p < 0.01$ (vs control).

uterine infection,[13, 14] which would be relevant to preterm delivery. Regardless of source, PAF could then stimulate intrauterine prostaglandin production, as it is known to do in other tissues,[15, 16] and thus be of focal importance in the process of labor. It is also possible that PAF may be produced by decidual macrophages activated as part of the process of labor.[20] Thus increased PAF production would be indicative of the process of labor, rather than an initiator of it.

Our results suggest that PAF affects prostaglandin production by the intact fetal membrane in a complex manner. PAF stimulated a small increase in the production of PGE_2 from the amnion but had a more potent effect on the production of both PGE_2 and PGE_2 metabolites from the intact fetal membrane and chorion-decidua. It would therefore seem that most of the PGE (PGE_2 plus metabolites) produced by the stimulated intact fetal membrane originates in the decidua, because the amnion alone contributes 10 to 20 ng/ml/24 hours (Fig. 1) compared with 1000 to 2000 ng/ml/24 hours (Fig. 2, *B*) from the intact membrane. This is consistent with other studies that indicate that most of the PGE produced by intact fetal membranes is detected as inactive metabolites[19, 21] and may originate in the decidua. In addition, PAF added to the decidual side of the intact fetal membrane stimulated PGE_2 production to a greater extent than PAF added to the amnion side of the membrane, which is consistent with other reports implicating the decidua in prostaglandin production during labor.[20, 22]

Intact fetal membranes obtained after labor showed a higher basal level of PGE_2 production, especially on the amnion side (Fig. 3), which is consistent with other reports indicating increased production of prostaglandins by amnion after labor.[23, 24] The addition of PAF to the amnion side of the membrane had no effect, whereas PAF added to the decidual side stimulated PGE_2 production by sixfold to tenfold on both sides of the membrane (Fig. 3). These results suggest that, although PAF can increase PGE_2 production by the amnion, the major effects of PAF are on the decidua when the intact fetal membrane is used. In addition, PAF stimulated very high levels of PGE_2 production after labor, which suggests that it may interact with factors in the fetal membrane after labor to stimulate prostaglandin production. Further studies are under way to assess which of the cell types present in the decidua (macrophages, stromal cells, and lymphocytes)[25] respond to PAF by increasing PGE_2 production.

These results indicate that PAF per se does not initiate the process of labor but rather may be an important factor in the maintenance of the elevated prostaglandin production integral to the process of labor. Further studies are needed to identify the factor or factors that act in synergism with PAF to increase fetal

membrane prostaglandin production. PAF added to the amnion side of the intact fetal membrane had little effect on PGE_2 production, which suggests that PAF in amniotic fluid, which may be fetal in origin, may have only a limited role in the initiation of labor. PAF produced by activated decidual macrophages may therefore be involved in the process of labor, so the control of the synthesis of PAF by these cells requires further investigation.

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Papanicolaou smear abnormalities in ambulatory care sites for women infected with the human immunodeficiency virus

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OBJECTIVE: We attempted to determine the prevalence of cervical dysplasia in human immunodeficiency virus–infected women in ambulatory care settings and to correlate Papanicolaou smears with demographic and clinical variables.

STUDY DESIGN: Papanicolaou smears of 135 women attending three ambulatory care clinics were reviewed. Chart review identified demographic and clinical variables, including CD4 count. Prevalence of abnormal smears was compared with baseline community rates. Demographic and clinical variables were correlated with Papanicolaou results with the χ^2 test.

RESULTS: Fivefold to eightfold increased rates of abnormal smears in human immunodeficiency virus–infected women were observed. Prevalence of abnormal smears increased from 21% in women with CD4 counts >600/mm³ to 45% in women with CD4 counts <400/mm³. Age, ethnicity, or mode of human immunodeficiency virus transmission was not significantly correlated with Papanicolaou smear findings.

CONCLUSION: Increased rates of abnormal Papanicolaou smears and significant correlation with CD4 counts were observed in human immunodeficiency virus–infected women at ambulatory care sites. We recommend comprehensive gynecologic care, including semiannual Papanicolaou smears, for all human immunodeficiency virus–infected women. (Am J Obstet Gynecol 1992;166:1232-7.)

Key words: Human immunodeficiency virus, acquired immunodeficiency syndrome, cervical cytology, Papanicolaou smears, CD4 lymphocytes

Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) are now increasing more rapidly in women than in men in the United States.[1] Conditions unique to women, notably gynecologic conditions, are beginning to gain recognition. However, because of the focus on HIV treatment in the setting of infectious disease specialization, women's primary care needs, including gynecologic care, have received less attention than they should.

Cervical dysplasia is of particular concern because of its potential for malignant development. Given that cervical neoplasia is known to be more common and more aggressive in iatrogenically immunodeficient women such as renal transplant patients,[2] cervical Papanicolaou test screening and information on cervical dysplasia are of particular importance in the growing population of women with HIV-associated immunodeficiency.

This study was stimulated by the concern of primary care providers in three different ambulatory sites for HIV-infected women. Subjective impressions of a high frequency of abnormal smears and genital warts among our patients and early reports of associations between cervical neoplasia and HIV disease[3-5] led us to initiate this multicenter study of cervical cytologic results and immunodeficiency. The goal of this study was to ascertain the prevalence of cytologic abnormality in HIV-infected women and to explore the relationships between associated variables including mode of HIV transmission, demographics, immune status, and HIV disease stage.

Methods

Three ambulatory care facilities offering comprehensive primary care to HIV-infected persons initiated a program of consistent gynecologic services and gathered routine gynecologic data for comparison. The sites were The Women and Children with AIDS Project at Cook County Hospital, Chicago, the Infectious Disease Clinic at Kings County Hospital, Brooklyn, and the Community Health Project, a satellite facility of Bellevue Hospital, New York.

At each of the three sites a protocol for routine gynecologic care was introduced that called for pelvic examination with Papanicolaou test and screening for sexually transmitted diseases (STDs) every 6 months. All women were informed of the protocol and the rationale

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6/1/34740

Table I. Distribution of subjects by ambulatory care site and other variables

Variable	*Kings County Hospital*		*Community Health Project*		*Cook County Hospital*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No. of patients	35		35		65		135	
Age (yr)								
<30	11	31	9	26	21	32	41	30
30-39	17	49	23	66	35	54	75	56
≥40	7	20	3	9	9	14	19	14
Race or ethnicity								
African American	27	77	9	26	36	55	72	53
Latina	8	23	4	11	13	20	25	19
European American	—	0	21	60	15	23	36	27
Mode of HIV transmission								
Intravenous drug use	9	26	24	69	42	65	75	56
Heterosexual intercourse	22	63	10	29	17	26	49	36
Transfusion	2	6	—	—	3	5	5	4
CD4 count (cells/mm^3)								
>600	—	—	6	17	17	26	23	17
400-599	4	11	8	23	12	18	24	18
200-399	10	29	10	29	11	17	31	23
<200	20	57	11	31	25	38	56	41
Active STDs								
Syphilis	2	6	1	3	2	3	5	4
Gonorrhea	2	6	1	3	0	0	3	2
Chlamydia	Not done		3	9	0	0	3	3

Note: Numbers may not sum to 100% because of missing data.

for its use and were offered gynecologic services at the clinic sites.

Pelvic examinations were performed and specimens were obtained for cervical cytologic testing (Papanicolaou test). Serum syphilis screening and gonorrhea culture were obtained in all cases; chlamydia assay and wet mount for *Candida* and trichomonas identification were obtained as indicated at each site.

Patients' charts were reviewed to obtain data on age, race or ethnicity, test results for chlamydia, gonorrhea, and syphilis, the original cytology report, mode of HIV transmission, stage of HIV disease, and CD4 lymphocyte counts obtained within 2 months of cytologic testing. Race or ethnicity was defined as African American, Latina, and European American with each category including both those born in the United States and immigrants.

Cytology slides were read by each institution's cytology staff who were unaware of the subjects' HIV status. Slides were reviewed by a fourth independent cytologist experienced in interinstitutional studies. Several slides from women with unknown HIV status were included to ensure a blinded review. The reviewer was not aware of the original cytologists' reports. The interpretation of the reviewer was used for the 113 slides reviewed, whereas the original interpretation was used for the 22 slides not available for review.

Smears were categorized according to the Bethesda system.[6] Slides were considered to show abnormality if they were reported as containing atypia of uncertain significance, low-grade squamous intraepithelial lesions (human papillomavirus, mild dysplasia), or high-grade squamous intraepithelial lesions (moderate and severe dysplasia, carcinoma in situ). Papanicolaou tests indicating inflammatory atypia or infection by any organism other than human papillomavirus were categorized as normal.

A baseline rate for "abnormal" Papanicolaou tests was obtained from the cytology service of each site. In each case this baseline rate consisted of the rate of abnormal Papanicolaou tests for outpatients attending routine medical, gynecologic, and obstetrics clinics. The baseline estimate did not include hospitalized patients or those attending specialty clinics such as oncology clinics.

Differences in proportions were estimated by the χ^2 test and the Fisher exact test. The degree of concordance between original and reviewed cytologic results was estimated with the κ statistic and the degree of skewness by a modified McNemar test for symmetry.

Results

The ambulatory care sites varied considerably with respect to race or ethnicity, mode of HIV transmission, and severity of immunodeficiency as judged by CD4 lymphocyte count (Table I). There was no difference with respect to age. Kings County Hospital subjects differed from those at Community Health Project and Cook County Hospital in having more severe immunodeficiency as measured by CD4 counts and in that only one quarter had been intravenous drug users. Community Health Project subjects differed from those

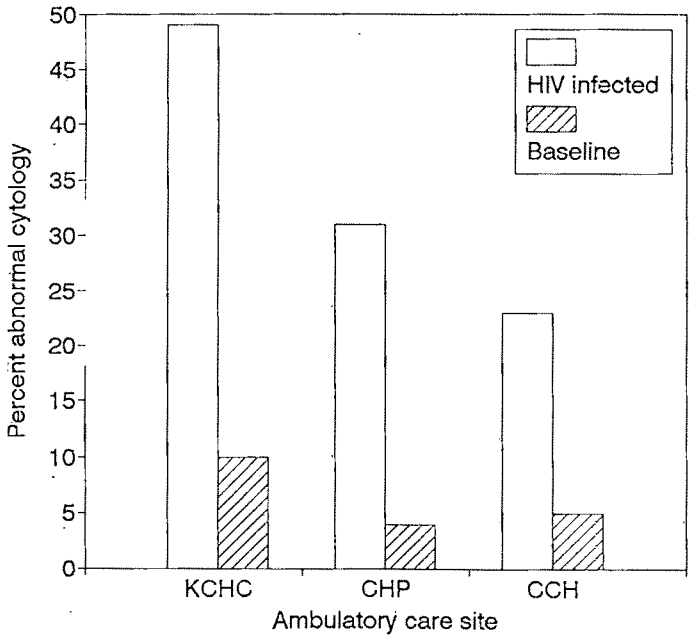


Fig. 1. Percent of HIV-infected women with cytologic abnormality compared with baseline rate at each ambulatory care site. *KCHC*, Kings County Hospital Center; *CHP*, Community Health Project; *CCH*, Cook County Hospital.

at Kings County Hospital and Cook County Hospital in that 60% were European American.

The frequency of abnormal smears was compared with the baseline rate at each institution according to the original criteria of each laboratoy (Fig. 1). In HIV-infected women the rate of abnormal Papanicolaou test results was 23% at Cook County Hospital, 48% at Kings County Hospital, and 32% at Community Health Project. While the baseline rate varied with each site, the rates in HIV-infected women were consistently fivefold to eightfold higher than the corresponding baseline rates.

Cytology slides were reviewed for 82% of Cook County Hospital subjects, 86% of Community Health Project subjects, and 86% of Kings County Hospital subjects. With the classification of normal versus abnormal, there was complete concordance between reviewer and original at Kings County Hospital. For Community Health Project two smears were reclassified from normal to abnormal, but the κ statistic of 0.84 indicated excellent agreement beyond chance. For Cook County Hospital eight slides were reclassified from normal to abnormal and two from abnormal to normal, and the κ statistic of 0.56 indicated fair to good agreement beyond chance. Overall, there was a significant tendency ($p = 0.02$ in McNemar's symmetry test) for the reviewer to upgrade normal results to abnormal ones.

The relation between selected patient characteristics and the frequency of cytologic abnormality is shown in Table II. There was no significant difference in the proportion of abnormal smears by age, race or ethnicity, ambulatory care site, or mode of HIV transmission. A difference that approached, but did not reach, statistical significance was that none of the five women with transfusion-acquired HIV infection had cytologic abnormality ($p = 0.08$ in Fisher exact test).

Overall, 41% of 134 subjects had CD4 counts $<200/mm^3$, 23% had counts of $200/mm^3$ to $399/mm^3$, 18% had counts of $400/mm^3$ to $599/mm^3$, and 17% had counts $>600/mm^3$. Women with CD4 counts $<400/mm^3$ had nearly a twofold higher prevalence of cytologic abnormality than women with higher CD4 counts (45% vs 26%). Although CD4 counts >600 were associated with the lowest proportion of abnormal smears, CD4 counts <200 were associated with the same rate of abnormal smears as CD4 counts of 200 to 399 (Fig. 2).

The proportion of women with both AIDS and cytologic abnormality was higher, but not significantly, than the proportion of HIV-infected women without AIDS but with cytologic abnormality. There was no difference in abnormality rates between those with symptomatic HIV disease and those without symptoms. In this series only six women had cytologic results indicative of high-grade squamous intraepithelial lesions. Of the six, four had CD4 counts <200 and two had CD4 counts between 200 and 399.

Low rates were found for active syphilis, gonorrhea, and chlamydia: 5 of 135 (4%) required treatment for

Table II. Correlation of cytologic abnormality with patient and HIV disease characteristics (n = 135)

Characteristic	*Total*	*Cytologic abnormality*		*Significance*
		No.	*%*	
Age (yr)				
<30	41	18	44	NS
30-39	75	27	36	
≥40	19	6	32	
Race or ethnicity				
African American	72	28	39	NS
Latina	25	9	36	
European American	36	13	36	
Site				
Kings County Hospital	35	17	49	NS
Community Health Project	35	13	37	
Cook County Hospital	65	21	32	
Mode of HIV transmission				
Intravenous drug use	75	30	40	NS
Heterosexual intercourse	49	20	41	
Transfusion	5	0	0	
Unknown	6	1	17	
Stage of HIV disease				
Asymptomatic	70	24	34	NS
Symptomatic	32	11	34	
AIDS	33	16	48	
CD4 count (cells/mm^3)				
>400	47	12	26	$p < 0.05$*
<400	87	39	45	

NS, Not significant.
*χ^2 test, 1 *df*.

syphilis, 3 (2%) of 135 for gonorrhea, and 3 of 100 (3%) for chlamydia.

Comment

Two significant findings emerge from this study. First is the high rate of cytologic abnormality compared with baseline rates in each of three demographically and geographically distinct ambulatory care centers. Second is the association between severity of immunodeficiency and frequency of cytologic abnormality.

Since this study was initiated, the association between HIV infection and cytologic abnormality has been confirmed in several studies[7-9] with odds ratios of about 5. Our finding of a fivefold to eightfold increase in cytologic abnormality in HIV-infected women over the baseline rate at each site is consistent with these other studies.

Two prior studies suggest that increased rates of cervical neoplasia are associated with increased immunodeficiency. Maiman et al.[10] found increasing rates of histologically confirmed cervical intraepithelial neoplasia as CD4 count decreased in 32 HIV-infected women. Schafer et al.[7] showed an association between lowered CD4 counts and increased prevalence of cytologic abnormality. Their results were similar to ours in the finding of a significantly higher prevalence of Papanicolaou test abnormalities in women with CD4 counts <400/mm^3 but no significant difference in abnormality rates within subgroups with CD4 counts <400/mm^3.

Several explanations for the association between HIV infection and cytologic abnormality may be proposed. Most cytologic abnormality is associated with infection by human papillomavirus, and human papillomavirus infection may have a more aggressive course in HIV-infected women than in immunocompetent individuals. It is known that immunosuppression in HIV-infected men and women places them at risk for unusually frequent or severe manifestations of certain other viral infections, for example, cytomegalovirus and herpes simplex virus. Increased frequency of human papillomavirus and associated anogenital neoplasia has been reported in HIV-infected men.[11, 12] Other immunodeficiency states resulting from congenital and iatrogenic factors are also associated with high rates of cervical neoplasia.[2, 13] In addition, an association between HIV infection and cervical dysplasia would be expected on the basis of common sexual risk factors since both cytologic abnormality and HIV infection are associated with having multiple sexual partners. In this study we did not have information on the sexual behavior histories of the women and therefore could not control for sexual behavior.

There is a growing consensus among clinicians that Papanicolaou test screening for cervical intraepithelial neoplasia must become a routine part of care for HIV-infected women and that treatment for human papil-

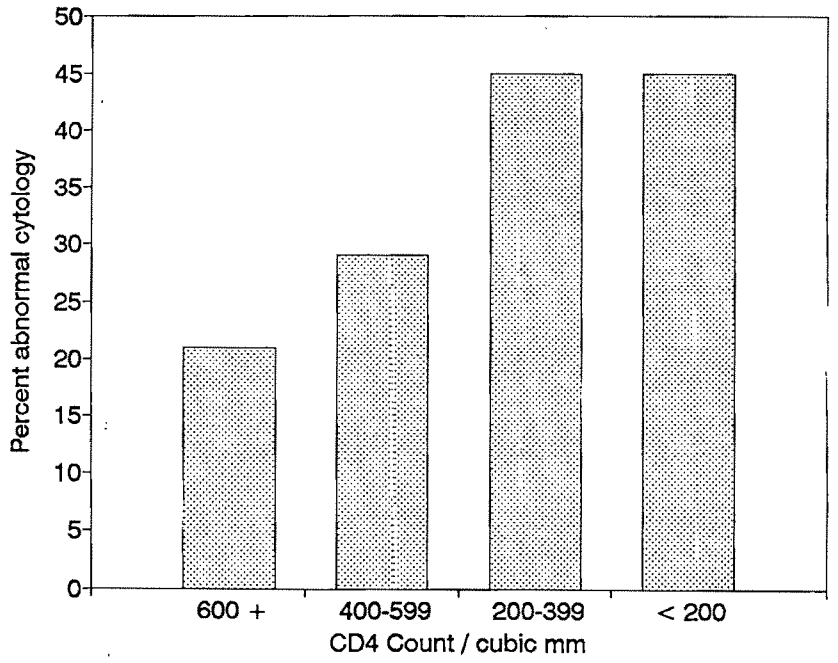


Fig. 2. Percent of cytologic abnormality by CD4 count. (Cytologic results reviewed by independent reviewer.)

lomavirus–related disease should be at least as careful as, or even more aggressive, than for uninfected women. There is still little information on the natural history of cervical disease in HIV-infected women on which to base guidelines for the proper interval for screening.[14] However, because of the indication that cervical disease may be more aggressive in the presence of HIV infection[13] and because of possible disordance between cytologic and histologic results,[10] we recommend that HIV-infected women have Papanicolaou tests at 6-month intervals rather than annually until the appropriate interval is clearly established. In addition, we recommend that all women with both low-grade and high-grade squamous intraepithelial lesions (grades 1 to 3 cervical intraepithelial neoplasia) on cytologic test results be referred for immediate colposcopy for the same reasons. The treatment of choice for cervical abnormalities in HIV-infected women has yet to be investigated. However, close and frequent follow-up after standard treatment is advisable because of the potential for recurrence.

The purpose of Papanicolaou test screening is to identify and treat cervical neoplasia at an early stage, preventing the development of invasive cancer and associated mortality. The course of cervical neoplasia in HIV-infected women must be evaluated since the immunodeficiency that allows a high prevalence of cervical disease may also allow rapid progression and poor response to therapy. As treatment of HIV-associated conditions becomes more effective and the lives of HIV-infected women are prolonged, the need to prevent the development of life-threatening cancer becomes more critical.

The clinical implications for women are clear. First, infectious disease specialists and other clinicians caring for HIV-infected women should ensure that their patients receive appropriate gynecologic care. Second, in areas with high rates of HIV disease, women with abnormal Papanicolaou test results should be considered at risk for HIV infection and should be offered confidential HIV counseling and testing.

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Intravenous nitroglycerin for uterine relaxation of an inverted uterus

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Intravenous nitroglycerin has recently come to the attention of obstetricians as a potent uterine relaxant. In the case presented, it was used to permit replacement of a tightly contracted, inverted uterus. Relaxation was rapid, effective, and associated with no adverse side effects. (AM J OBSTET GYNECOL 1992;166:1237-8.)

Key words: Nitroglycerin, uterine inversion

Since the first synthesis of nitroglycerin in 1846, it has found many uses among specialists in internal medicine. These include therapy for acute episodes of angina, ischemic heart disease, unstable angina syndrome, congestive heart failure, and control of acute hypertension during cerebrovascular and coronary surgery. The safety, predictability, and ease of intravenous administration of this drug have been firmly documented. In recent years intravenous nitroglycerin has come to the attention of the obstetrician as a potent uterine relaxant. This case describes its use to enable replacement of a completely prolapsed, inverted uterus.

Case report

The patient was a 27-year-old Chinese woman, gravida 3, para 1, abortus 1, admitted at 40 weeks of gestation with onset of labor. Prenatal course was significant for gestational diabetes controlled by dietary measures. Medical and surgical history was negative. The patient was admitted in active labor at 4 cm dilatation with contractions every 10 minutes. Artificial rupture of membranes was performed; over the next 4 hours the cervix progressed to full dilatation, and she was taken to the delivery room. Blood pressure throughout labor was 100/70 mm Hg. Normal spontaneous vaginal delivery ensued over a right mediolateral episiotomy. With traction on the cord and Brandt maneuver to secure the uterus, delivery of the placenta was attempted. With the bulk of placenta at the introitus the patient appeared to be in an unusual amount of pain for delivery of the placenta. It was then noted that the uterus was external to the introitus. The placenta was tightly adherent to the inverted, completely prolapsed uterus.

It was decided to attempt uterine relaxation with intravenous nitroglycerin, as opposed to general anesthesia. Nitroglycerin 50 μg was administered intravenously, and within 90 seconds moderate uterine relaxation was noted but it was not sufficient to effect replacement of the uterus. Sedation with 10 mg keta-

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mine given intravenously was also used. An additional 50 μg dose of nitroglycerin was administered, and complete uterine relaxation was achieved. The placenta was easily stripped from the endometrium, and the uterus was replaced with the assistance of the operator's fist. The relaxation was reversed within 2 minutes by uterine massage, intravenous oxytocin, and intramuscular ergotrate, and the uterus contracted. Episiotomy was then repaired. Vital signs were monitored by an automated blood pressure cuff and electrocardiogram. Blood pressure was 90/60 mm Hg and pulse 130 beats/min with uterine inversion, 95/55 mm Hg and 140 beats/min after the initial dose, 85/55 mm Hg and 130 beats/min after the repeat dose, and 100/55 mm Hg and 130 beats/min on transfer to the recovery room. There was a blood loss of 1800 ml that necessitated transfusion.

Comment

Uterine inversion, a serious and often unexpected obstetric complication, has a reported incidence of 1 in 2000 deliveries. Inversion is associated with excessive hemorrhage and shock. Traditional management includes prompt replacement of the uterus, vigorous hydration and transfusion, and often the use of general anesthetics to provide relaxation of the pregnant uterus. This course has significant risks, especially in pregnancy, where the risk of aspiration of gastric contents is markedly increased. Halothane is a cardiodepressant and is often associated with hypotension.

Nitroglycerin has been shown to be an effective relaxant of strips of rat myometrium in vitro, but there are few reports of the use of intravenous nitroglycerin for uterine relaxation in humans. Peng et al.[1] reported their experience with intravenous nitroglycerin for manual removal of the placenta in 15 patients where the cervix had contracted. In that report a dose of 500 μg was used, with success in all cases. However, at that dosage a statistically significant, although clinically unimportant, reduction in blood pressure was noted. Side effects such as headache and prolonged uterine relaxation were not observed.

In another report, DeSimone et al.[2] described the use of a 50 to 100 μg dose in 22 patients who required uterine muscle relaxation for manual removal of the placenta when the uterus was firmly contracted around the cord. In all cases relaxation was achieved with no clinically significant changes in blood pressure or heart rate. No patient complained of headache, palpitations, or other subjective effects.

This is the first reported case of intravenous nitroglycerin for use in patients with an inverted uterus. It is ideal for such a situation because of its powerful uterine relaxant effect and short plasma half-life (2 minutes). With the low doses of nitroglycerin used, the degree and duration of uterine relaxation can be controlled easily and without maternal side effects. In this way the significant risks of general anesthesia may be circumvented.

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Severe anti-C hemolytic disease of the newborn

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OBJECTIVE: Because of referral of a C-alloimmunized pregnant woman with a previous hydropic death whose fetus survived after four intraperitoneal transfusions, prevalence and severity of anti-C hemolytic disease of the newborn were investigated.
STUDY DESIGN: The numbers of C- or Ce-alloimmunized pregnancies in Manitoban women and their outcome for the 28-year period ending Oct. 31, 1990, were reviewed. The literature relating to C or Ce alloimmunization from 1944 to 1990 was surveyed.
RESULTS: In Manitoba for the period reviewed there were 120 pregnancies in 80 C- or Ce-alloimmunized women. Twenty-two ended in abortion and two in fetal death unrelated to anti-C or anti-Ce. Of the remaining 96, 33 fetuses of 32 pregnancies were affected but only eight (6.7%) required treatment after birth. None were severely affected. In the literature there are only three other reported deaths from C or Ce hemolytic disease; two of the three may have been the same patient. The prevalence of C or Ce alloimmunization reported in various series, including our own, ranged from 8.7 to 185 per 100,000 pregnancies.
CONCLUSIONS: Because on rare occasions, C or Ce alloimmunization can cause severe hemolytic disease, criteria for investigative measures such as amniocentesis or cordocentesis do not differ from the criteria for instituting these measures in Rh_o (D)–alloimmunized pregnancies. (Am J Obstet Gynecol 1992;166:1239-43.)

Key words: Anti-C hemolytic disease, fetal transfusions

Although anti-C is listed as an antibody capable of causing severe hemolytic disease of the newborn,[1] reports of anti-C causing severe hemolytic disease are extremely rare. In 1986 the Rh Laboratory and the Division of Fetal and Maternal Medicine, Faculty of Medicine, University of Manitoba, and the Health Sciences Centre, Winnipeg, participated in the management of an anti-C alloimmunized pregnant women whose fetus required intrauterine transfusions to survive. In 46 years of experience with maternal alloimmunization, this was the Rh Laboratory's first example of anti-C hemolytic disease of such severity. We report this case in detail, with a review of the Rh Laboratory experience and the experience of others with anti-C or anti-Ce alloimmunization.

Case report

At the time of her first pregnancy this para 2, gravida 3, group A, Rh-positive, C-negative, Cw-negative, e-negative (R^2R^2) woman was transfused at cesarean section for abruptio placentae. Her second pregnancy ended at 37 weeks' gestation with a hydropic stillbirth. At that time she was found to have very strong anti-C and anti-Cw and very weak anti-e blood group antibodies. Her husband was group B, Rh positive, C positive, e positive, and Cw negative (R^1R^1). Thus anti-C was the alloantibody responsible for the hydropic fetal death.

Mrs. S. was referred to Winnipeg in 1986, in her third pregnancy at 22½ weeks' gestation. The anti-C titer was 256 in albumin and 1 in saline solution. Two amniocenteses had been carried out at the referring center, the Δ optical density readings at 450 nm (ΔOD_{450}) were 0.355 and 0.362 at 20½ and 22½ weeks' gestation. Two days later in Winnipeg, at the time of the first intraperitoneal fetal transfusion, the ΔOD_{450} reading was 0.346. Hepatomegaly was evident on ultrasonography, but the fetus was not hydropic.

Four intraperitoneal fetal transfusions were carried out with infusion of tightly packed, fresh, compatible, group O, C-negative, Cw-negative, e-negative (R^2R^2), Kell-negative red blood cells at 22½, 24, 27½, and 31 weeks' gestation.

She was delivered of a female infant by elective cesarean section at 33½ weeks' gestation in the tertiary-level center from which she had been referred. Jaundice was present and there was marked hepatosplenomegaly. Cord red blood cells were 99.7% adult donor in origin by Kleihauer acid elution testing. Cord hemoglobin level was 117 gm/L; cord serum bilirubin level was 138 μmol/L total and 38 μmol/L by direct reaction.

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6/1/34297

Table I. Anti-C or anti-Ce alloimmunization, Manitoba, Nov. 1, 1962, to Oct. 31, 1990
475,000 pregnancies

Anti-C or anti-Ce immunized women	*Total anti-C or anti-Ce pregnancies*	*Abortions and unrelated losses*	*Unaffected (direct antiglobulin test–negative) infants*	*Affected, no treatment required*	*(Direct antiglobulin test–positive) treatment required**	*Perinatal or intrauterine death caused by anti-C and anti-Ce*
80	120†	24‡	64	25§	8‖	0

*Treatment: exchange transfusion and/or phototherapy.

†Fifty of the 120 were not detectable by an indirect antiglobulin test.

‡Twenty-two spontaneous or induced abortions, two fetal deaths unrelated to anti-C or anti-Ce. Both women had subsequent healthy surviving infants.

§One set of twins, 24 pregnancies.

‖One woman accounted for four of the eight babies.

An immediate double-volume exchange transfusion was carried out, followed by a second exchange transfusion on the second day of life. The highest indirect serum bilirubin level was 228 μmol/L (13.4 mg/dl). She had evidence of transient hepatocellular damage,[2,3] an index of severe hemolytic disease, with the direct (conjugated) bilirubin level peaking at 125 μmol/L. The neonatal nursery course was uneventful. At 7 months of age she was progressing normally.

Results

Prevalence of anti-C or anti-Ce alloimmunization. The Rh Laboratory records for the 28-year period, Nov. 1, 1962, to Oct. 31, 1990, were reviewed. The laboratory is responsible for antibody screening of all women in Manitoba (population of 1 million with 17,000 pregnancies annually). The policy of the laboratory is that all pregnant women should be screened at their first prenatal visit. Unimmunized Rh-negative women are then screened at monthly intervals. A second blood sample for screening of unimmunized Rh-positive women is requested at 28 to 34 weeks' gestation but is not always obtained.

Antibody screening of maternal serum is carried out by a sensitive two-stage, enzyme-treated red blood cell method[4] and a low-ionic autoanalyzer method.[5]

If an Rh-positive pregnant woman has a blood group alloantibody, further blood samples are requested at 2- to 8-week intervals, depending on length of gestation, the specificity of the antibody, and its strength (titer). If the antibody is one that is known to cause fetal hemolytic disease, amniotic fluid spectrophotometry or, in the past 5 years, fetal blood sampling is carried out, on the basis of the same criteria used for initiating either procedure in Rh-negative, Rh_o (D)–immunized pregnant women (history of a prior perinatal death, a fetus or infant requiring treatment, or antibody titers of ≥16 in albumin or ≥32 to 64 by indirect antiglobulin).[6]

Because the Rh Laboratory carries out all cord blood testing and treatment of affected babies after birth and, in conjunction with the Division of Fetal and Maternal Medicine, carries out all amniotic fluid measurements, fetal blood testing, and fetal transfusions in Manitoba, the subsequent course of each pregnancy and the fetal-neonatal outcome are known and are part of each alloimmunized woman's Rh Laboratory record.

The numbers of anti-C or anti-Ce alloimmunized pregnancies, excluding those in Rh-negative women with anti-D, for the 28-year period ending Oct. 31, 1990, are set out in Table I, with their respective outcomes. There were 80 C- or Ce-alloimmunized women who had 120 pregnancies (121 conceptuses) in the 28-year period. Nineteen women, six of whom were Native Americans, had 59 of the pregnancies (60 conceptuses). Of the remaining 61 women who had one C- or Ce-alloimmunized pregnancy, only three were Native Americans.

There were 22 abortions and two fetal deaths unrelated to C or Ce alloimmunization in the study group. Of the remaining 96 cases, 33 fetuses of 32 pregnancies (27.5%) were affected. However, only eight fetuses (6.7%) required treatment after birth: Two needed exchange transfusions and six required only phototherapy. Two of the six subsequently required simple transfusions.

All eight treated babies were only moderately affected. None required induced early delivery and none required intrauterine fetal transfusion. In this series there were no deaths caused by C or Ce alloimmunization. One woman, a Native American, accounted for four of the eight babies who needed treatment (one required exchange transfusion and the other three required phototherapy only). Her next baby, again affected, did not need treatment. In all, she accounted for seven of the 120 C- or Ce-alloimmunized pregnancies.

Some Manitoban C-alloimmunized women had anti-C or anti-Ce antibody titers as high as that of our patient (256 in albumin, 1 in saline solution). Five women with affected babies who required treatment had anti-C titers of 128 in albumin and 8 in saline solution, 64 in albumin and 8 in saline solution, 64 in albumin and 32 in saline solution, 16 in albumin and 16 in saline so-

Table II. Surveys that include C and Ce maternal alloimmunization

Author	Pregnant population	No. with anti-C or anti-Ce		Affected offspring		Babies requiring treatment		Death from hemolytic disease of fetus and newborn
		No.	Per 100,000 pregnancies	No.	No. per 100,000 pregnancies	No.	Proportion (% of whole)	
Hardy and Napier[8]	507,720	44	8.7	25	4.9	3	6.8	2*
Clarke et al.[9]	6-7 million			Perinatal mortality report only				1*
Bowell et al.[17]	280,000	38	13.6	5	1.8	1	2.6	0
Pepperell et al.[18]	72,138	9	12.5	4	5.5	2	22.2	0
Polesky[19]	43,100	5	11.6	Hemolytic disease of fetus and newborn not described				—
Queenan et al.[20]	18,378	34	185	2	10.9	1	2.9	0
Spielman and Seidl[21]	16,643	0		0		0		0
Beal[22]	41,078	5	12.2	Hemolytic disease of fetus and newborn not described				—
Zuliani et al.[23]	9,525†	2	21.0	0		0		0
Solola et al.[24]	5,559	0		0		0		0
Giblett[25]	6 yr Seattle	4		2	(Degree of hemolytic disease of fetus and newborn not described)			—
Current investigation	475,000	120‡	25.3	33	6.9	8§	6.7	0

*One of the deaths in the series of Hardy and Napier[8] may also be the one in the series of Clarke et al.[9]

†Highly selected referred population.

‡One set of twins, therefore 121 conceptuses; 50 of the 120 had enzyme-only detectable antibodies.

§One woman accounted for four of the eight babies.

lution, and 8 in albumin and 0 in saline solution, respectively. Three women who had mildly affected babies not requiring treatment had anti-C titers of 2048 in albumin and 512 in saline solution, 256 in albumin and 16 in saline solution, and 256 in albumin and 8 in saline solution, respectively.

Literature survey. The literature relating to anti-C or anti-Ce alloimmunization was surveyed from 1944 to 1990 (*Quarterly Cumulative Index Medicus*, 1944 to 1956; *Current List of Medical Literature*, 1950 to 1959; and *Cumulated Index Medicus*, 1960 to 1990). Although the review of the non-English literature was thorough, it may not have been complete; the review of the English literature was comprehensive.

Perinatal deaths from anti-C or anti-Ce hemolytic disease. Reports of perinatal deaths or fetuses requiring transfusions for anti-C or anti-Ce hemolytic disease are extremely rare. In 1981 Baker et al.[7] reported on a group A, Rh-positive, C-negative woman in whom anti-C developed as a result of blood transfusion in childhood. Her first affected baby required phototherapy. Her second pregnancy ended at 39 weeks' gestation in a macerated stillbirth. There was autopsy evidence of erythroblastosis. The anti-C titer at that time was 1024 in albumin. At the beginning of her third pregnancy, the anti-C titer was 512 in albumin. Because of amniotic fluid bilirubin levels of 4.8 and 6.0 μmol/L at 27 and 29 weeks' gestation, three intraperitoneal fetal transfusions were carried out (90, 110, and 130 ml of C-negative compatible blood at 30, 32, and 34 weeks' gestation). The infant, delivered at 36 weeks' gestation, had a cord hemoglobin concentration of 162 gm/L (65 gm/L of which was residual fetal C positive in origin). He did well after phototherapy and one exchange transfusion.

Hardy and Napier[8] in their review of red blood cell antibodies in Rh-positive women in south and mid Wales over a 30-year period (1948 to 1978) described two infants with hemolytic disease caused by anti-C who died, one after an exchange transfusion and one without undergoing exchange transfusion. They gave no further details of these two cases. Clarke et al.[9] in their report of deaths from hemolytic disease in England and Wales in 1984 and 1985 report, without giving any details, one perinatal death from C hemolytic disease in the 9-year period from 1977 to 1985. It is quite possible

that the perinatal death reported by Clarke et al. is one of the deaths reported by Hardy and Napier.

Remaining case reports. All remaining case reports of anti-C or anti-Ce alloimmunization[10-17] described affected infants who did not require treatment or who needed only exchange transfusion or phototherapy. None were severely affected, other than that of Waller et al.[10] (1944). This, the first reported example of hemolytic disease caused by anti-C, was a baby born at 39 weeks' gestation who had moderately severe hemolytic disease and required seven simple transfusions. The syndrome of transient hepatocellular damage developed in the baby, but it recovered with no neurologic sequelae.

Atypical or irregular blood group antibody surveys. Various surveys of the prevalence of non–Rh_o (D) blood group alloimmunization, including anti-C and anti-Ce, have been published[8,9,18-25] (Table II). The prevalence of anti-C and anti-Ce alloimunization ranged from 8.7[8] to 185[20] per 100,000 pregnancies. In the current series the prevalence was 25.3 per 100,000 pregnant women (Table II).

The numbers of affected babies requiring treatment in all of the series reporting affected babies needing treatment totaled 15, eight of them in our series. The incidence of affected babies requiring treatment compared with the total number of anti-C and anti-Ce alloimmunized pregnancies ranged from 2.6% to 22.2% (5.7% in our series).

Comment

Anti-C, Ce alloimmunization occurs rarely and when it does occur does not often cause serious hemolytic disease. The reported prevalence of C and Ce alloimmunization in unselected pregnant populations ranges from 8.7 to 13.6 per 100,000 pregnancies, except in the series of Queenan et al.[20] and our series. We have no explanation for the extremely high prevalence of C alloimmunization in the series of Queenan et al. (185/100,000). The reasons for the increased prevalence in our series are twofold. First, a sensitive two-stage, enzyme-treated red blood cell screening method was used.[4] Forty-two percent of our C or Ce alloimmunized population had antibodies detected only by this method and not by indirect antiglobulin.[6] If indirect antiglobulin screening alone had been used, which may well have been the case in the other series, the prevalence of C or Ce alloimmunization in our series would have been 14.7 per 100,000 pregnancies. Second, the prevalence of anti-C or anti-Ce in Manitoban women has an overrepresentation of Native Americans, six of 19 such women (32%) accounting for 25 of the 59 multiple alloimmunized pregnancies (42%). There is no such overrepresentation in the 61 women who had only one C or Ce alloimmunized pregnancy (3 or 5%). If one corrects for the multiparity of Manitoban Native American C and Ce alloimmunized women, the prevalence of C or Ce alloimmunization becomes 12.2 per 100,000 pregnancies, well within the 8.7 to 13.6 per 100,000 pregnancies reported by others.

Although anti-C or anti-Ce alloimmunization very rarely causes perinatal deaths (four or five in all reported series in the past 46 years), it does so on occasion. We believe that the C or Ce alloimmunized pregnant woman should be managed in the same manner as the D, c, or Kell alloimmunized pregnant woman. It would appear from the case of Baker et al.[7] and our case that blood transfusion causing C alloimmunization is a significant risk factor.

From the standpoint of the critical anti-C or anti-Ce antibody titer at which invasive, investigative procedures such as amniocentesis or cord blood sampling should be carried out, our patient had a titer of 256 in albumin. The patient of Baker et al.[7] had a titer of 512 in albumin. These titers are considerably higher than the critical anti-D titers for similar investigations. Several of our patients with mildly or moderately affected babies had similar or even higher antibody titers, although most had greater immunoglobulin M (saline solution) components. However, because one of the women had an affected baby who required treatment in spite of a titer of 16 in albumin and 16 in saline solution and another had a titer of only 8 in albumin and 0 in saline solution, we recommend that the same titer criteria for invasive investigations in C or Ce alloimmunized pregnancies as in D alloimmunized pregnancies should be used, $\geq$16 in albumin and $\geq$32 to 64 by indirect immunoglobulin.

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An integrated therapy for peripartum pelvic instability: A study of the biomechanical effects of pelvic belts

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OBJECTIVES: The objectives of this study were to investigate the influence of pelvic belts on the stability of the pelvis and to discuss the treatment of peripartum pelvic instability.
STUDY DESIGN: In six human pelvis-spine preparations, sagittal rotation in the sacroiliac joints was induced by bidirectional forces directed at the acetabula. Weight-bearing was mimicked by the application of a compressive force to the spine. The biomechanical effect of a pelvic belt was measured in 12 sacroiliac joints.
RESULTS: The pelvic belt caused a significant decrease in the sagittal rotation in the sacroiliac joints. The effect of a 100 N belt did not differ significantly from that of a 50 N belt.
CONCLUSION: The combination of a pelvic belt and muscle training enhances pelvic stability. The load of the belt can be relatively small; location is more important. The risk of symphysiodesis, especially as a result of the insertion of bone grafts, is emphasized. (Am J Obstet Gynecol 1992;166:1243-7.)

Key words: Pelvic instability, low back pain, sacroiliac joint, symphysiolysis, symphysiodesis

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The pelvis must be considered part of a complex kinematic chain between the legs and spine. Important connections in this chain are the joints between the sacrum and the L-5 (intervertebral disc and zygapophyseal joints) and the sacroiliac joints. Several studies demonstrate mobility in the sacroiliac joints.[1-5] Profound effects on the kinematic performance of the spine result from exceptional laxity of the sacroiliac

joints, from which dysfunction of the low back, pelvis, and hip joints may occur.[5]

The reported incidence of pelvic instability, which is inadequately characterized, varies from 0.005% to 4%.[6, 7] This lack of characterization may be related to the lack of agreement for criteria that differentiate between normal and pathologic peripartum relaxation.[8]

Since 1930, a radiologic method has been used to diagnose pelvic instability.[9] However, because most symphyseal joints show peripartum diastasis, radiographs are of little value.[3, 10] Callahan[8] stated that without clinical findings even a 3 to 5 cm separation cannot be considered a rupture of the symphysis in the absence of clinical symptoms. Clinical evaluation seems to be the only method by which to discover whether symphyseal diastasis creates symptoms.

In most studies on pelvic instability, limited attention is given to the functional anatomic interaction of the constituent parts. Pelvic instability defined according to topographic anatomy can be viewed as abnormal displacement of the pubic bones and treated as an isolated problem. In that case it is easy to forget that hypermobility at the symphysis is exclusively possible with hypermobility in the sacroiliac joint and/or profound bone deformation. Although bone deformation cannot be disregarded, extensive deformation is not usual (Vleeming A, et al. Unpublished observations). Therefore hypermobility of the symphysis is accompanied by hypermobility in the sacroiliac joints.

One of the structural devices causing stability in the sacroiliac joints, as well as in other joints, is the shape of the articular surface. Recently, Vleeming et al.[11] demonstrated specific roughening patterns in the sacroiliac joints that were more pronounced in men than in women. As shown in a biomechanical study,[12] articular surfaces with roughening patterns show higher friction coefficients. Roughening of the sacroiliac joints, which increases stability, was considered to be a physiologic process that reflected dynamic development. With reference to a loading mode in the frontal plane, friction in the sacroiliac joints could theoretically be increased by the use of a pelvic belt.[12] In that biomechanical model the location of the belt, and not the load, appeared to be crucial.

The use of pelvic belts in peripartum women has been demonstrated by Snijders et al.[13] Furthermore strapping of the pelvis gave satisfactory results in cases of symphysiolysis.[14-17] However, there is no experimental evidence that shows how strapping of the pelvis is effectively performed; the specific biomechanical effects are also obscure. In this study the influence of pelvic belts on the stability of the pelvis has been evaluated by measuring the effect of the belt on sagittal rotation (nutation and contranutation) in sacroiliac joints of human pelvis-spine preparations. The requested precision of the measurements cannot be obtained in living persons unless invasive techniques are used.

Material and methods

Six human specimens were embalmed by vascular perfusion with a medium containing 2.2% formaldehyde. Four of the specimens (P2, P3, P4, and P5) were female (age range 83 to 97 years). The ages of the male specimens (P1 and P6) were 76 and 90 years, respectively. The fifth lumbar vertebra of each specimen was connected to a frame. To mimic a real-life situation, an artificial gravity force was applied to the pelvis (for details, see Vleeming et al.[18]). Bilateral forces of 150 N were applied to the acetabula of the iliac bones to induce movement in the sacroiliac joints. To induce nutation (flexion) and contranutation (extension), cranioventral and dorsal forces, respectively, were used. Sagittal movement between ilium and sacrum was recorded by digital displacement meters. Movement was measured with and without a pelvic belt. For this purpose a 110 × 5 × 0.3 cm leather pelvic belt was positioned just above the greater trochanter. The tension and the size of the belt could be tightened with adjustable hooks and a steelyard.

Movement in each of the 12 sacroiliac joints was tested at four subsequent stages: without belt, with a pelvic belt of 50 N tension, with a belt of 100 N tension, and without belt. Stage 4 served to control whether the sacroiliac joints remained intact during the experiment.

The amount of rotation was recorded during the first, fifth, and tenth minutes of force. The measurements at 1 and 5 minutes were used to control whether displacement occurred regularly. Normally, about 90% of total rotation occurred during the first minute. The measurements of the tenth minute (generally three measurements for each joint at each stage) were used to calculate the amount of rotation between sacrum and ilium. For the method of calculating total rotation (nutation plus contranutation), see Vleeming et al.[18] For each joint the significance of the differences between the stages of the experiment was tested with the Student t test; for all joints as a group the sign test was used ($p \leq 0.05$ for both). Because no significant differences occurred between the measurements of stage 1 and stage 4, those data were grouped and used as controls.

Results

Without a pelvic belt, the total rotation (nutation plus contranutation) in the sacroiliac joints varied between 0.12 and 4.54 degrees (Fig. 1). In seven out of 12 sacroiliac joints, application of the 50 N loaded pelvic belt caused a significant decrease in rotation. In these preparations the average reduction in rotation was 29.3% For all preparations together rotation was significantly

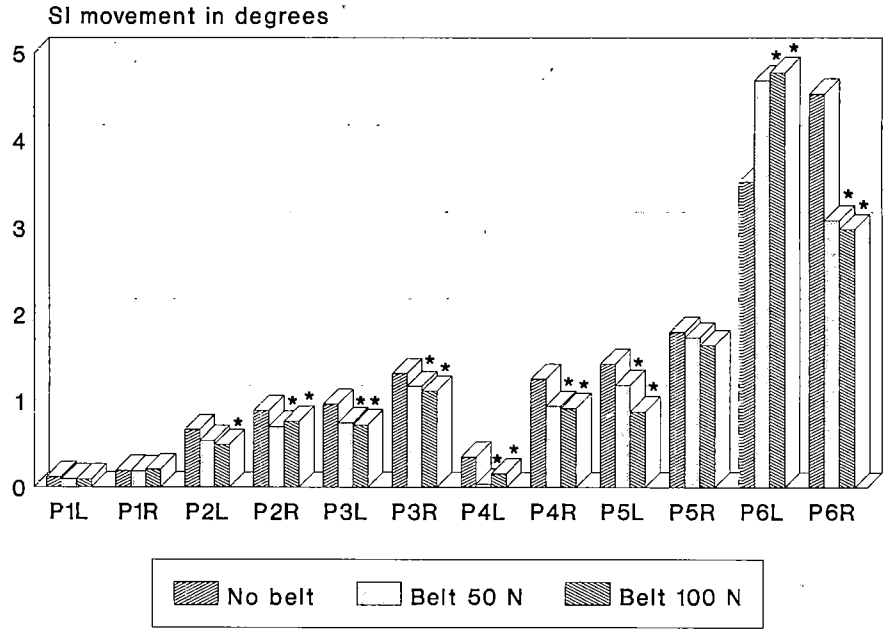


Fig. 1. Effect of application of 50 and a 100 N pelvic belts on sagittal rotation in 12 sacroiliac *(SI)* joints (*L*, left; *R*, right). *Asterisk*, Significant difference from control.

reduced by 18.8%. A comparable result was obtained with the 100 N belt: a significant decrease in eight sacroiliac joints and 29.5% and 18.5% reductions, respectively. Again, the reduction in rotation was statistically significant. The differences between the 50 and 100 N belts were not statistically significant.

The belt failed to reduce rotation in four sacroiliac joints, three of which were in male pelvises. One of these joints (pelvis 6, left), showed a significant increase in rotation.

Comment

Specific comment. In this study the effect of a pelvic belt on sagittal plane movements was studied in 12 sacroiliac joints. Movement was significantly restricted by application of a nonelastic belt with a tension of 50 N. Larger forces did not give better results; this was also the case in the previously mentioned biomechanical model,[12] in which the efficacy of a pelvic belt was primarily determined not by the amount of tension but by the site of application. Wearing the belt just above the greater trochanter achieved self-bracing of the pelvis. Increase of force in this instance may even be counterproductive because the symphysis is artificially compressed.

In one joint of a male specimen, rotation in the sacroiliac joints was increased by the belt. This reversed effect may reflect degeneration of the joint or damage to the sacroiliac joint by disintegration of ridges and depression.[11] Motion in the sacroiliac joints of female subjects was decreased by the belt. This decreased motion may be related to the relatively low friction in female sacroiliac joints that results from their relatively small and flat articular surfaces,[11] which, when combined with hormonal weakening of the pelvic ligaments, may result in unstable pelvic joints during pregnancy. Initiating higher friction by means of a pelvic belt can enhance stability of the sacroiliac joints and the symphysis. Then less force in the ligaments is needed for bearing the upper body.[12]

Hypermobility at the symphysis needs hypermobility in the sacroiliac joints. However, exceptions may occur. There is evidence that relaxin stimulates metabolic processes in both the symphysis and the sacroiliac joints,[17, 19] but in peripartum symphyses its effect differs markedly.[20] Whether the mobilizing effect of relaxin on the symphysis is in concert with the relaxation of the sacroiliac joints is unknown. As a consequence, relaxin could be responsible for the unbalanced relaxation of the sacroiliac joints and symphysis, which could result in isolated symphyseal or sacroiliac joint problems.

General comment. Symphysiolysis is frequently the diagnosis in cases of pelvic instability. Objective criteria are generally lacking. That diagnosis is often followed by treatment primarily directed at the symphysis, which may be assumed to be the site of the instability. However, this approach might preclude appropriate treatment. In the preparations studied an artificial separation of the pubic bones at the symphysis did not generate a massive loss of pelvic stability (Vleeming A, et al. Unpublished observations). These findings agree with those of Van Roosmalen.[21] These studies dem-

onstrate that true pelvic instability is characterized by simultaneous slackening of the dorsal sacroiliac joint ligaments and relaxation of the symphysis.

In a characteristic hospital report on symphysiodesis, the orthopedic surgeon stated, "A bone graft was inserted under strain in the symphysis." Insertion of bone grafts into the symphysis under strain as a treament for pelvic instability will lead to tension in the thin ventral sacroiliac ligaments and slackening of the dorsal interosseous sacroiliac joint ligaments, which will have serious consequences because the dorsal ligaments are extremely thick and are important to the stability of the sacroiliac joints.

If bone growth of the graft is achieved, another problem arises. The individual pelvic bones will be immobilized and will function as one massive bone with respect to the sacrum and vice versa. Increased hypermobility at the sacroiliac joints could result. Hypermobile sacroiliac joints imply large forces at the pubic bones. Thus insertion of bone grafts (especially under tension) into the symphysis may be counterproductive to a normal pelvis-spine mechanism. As Lavignolle et al.[3] demonstrated, the instantaneous center of rotation of the constituent bones of the pelvis is located at the symphysis. An appropriate functioning pubic articulation at the symphysis is therefore imperative for normal pelvic function.

Surgical symphysiodesis is based primarily on a study by Olerud and Walheim.[22] However, their relatively positive results may be partially the result of arthrodesis of the sacroiliac joints: Five of eight patients evaluated received both symphysiodesis and arthrodesis of the sacroiliac joints! Thus we question whether symphysiodesis is an appropriate treatment for pelvic instability, especially when bone grafts are used, because that approach disregards two facts: Nature created mobility between the pubic bones of men and women, and encroaching on the kinematic chain must have consequences elsewhere.

We concluded that a pelvic belt enhances pelvic stability because it reduces movement in the sacroiliac joints. If pelvic instability occurs, clinical tests can indicate whether problems are primarily at the sacroiliac joint, the symphysis, or both. Determination of the amount of instability in relation to pain seems to be crucial. Immediately after the initial functional test, the test procedure can be repeated with a pelvic belt, which can be initially positioned just above the greater trochanter. The exact position must be adjusted according to the patient's demands. If pain is diminished, therapeutic application of a pelvic belt is advisable; this supports the beneficial results of strapping the pelvis as described by several authors.[8, 14-17] In severe cases strapping can be combined with bed rest.[14, 15] If hypermobility at the symphysis occurs, the lithotomy position with strongly abducted legs must be used cautiously because of the adductor muscle stress on the symphysis.[15, 23, 24]

We do not advise use of the pelvic belts as a monotherapy. In our opinion simultaneous muscle training, especially training of the internal pelvic musculature and the gluteus maximus muscle, is important. This approach is based on a biomechanical study[12] and on the consideration that the sacrotuberous ligaments are important structures in the kinematic chain between the pelvis and spine. Muscles attached to these ligaments, such as the gluteus maximus and in some individuals the long head of the biceps femoris, can dynamically influence movement in the sacroiliac joints. The gluteus maximus in particular can enlarge friction in these joints and thus can stabilize the pelvis.[25] If the proposed integrated approach is not efficient, then an increase of belt tension is not recommended.

If none of these interventions gives beneficial results, a surgical approach can be considered: arthrodesis of the sacroiliac joints if the sacroiliac joints are exceptionally unstable and symphysiodesis if symptoms are located predominantly at the symphysis. In the latter case the two-hole plate technique described by Webb et al.[26] seems to be appropriate from a functional anatomic and biomechanical point of view.

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Giant ovarian cyst: Case report

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We present the case of a woman 22 years old with an ovarian cyst of 6 years' duration. The total weight of the tumor was 154 pounds (70 kg). She was treated surgically with good results. (Am J Obstet Gynecol 1992;166:1247-8.)

Key word: Giant ovarian cyst

We describe a case of a 22-year-old woman with an ovarian cyst of 6 years' duration weighing 154 pounds.

Case report

Clinical history. The patient was a 22-year-old woman admitted to the Woman's Hospital (Health Department) on Nov. 21, 1988. She came from a town in the state of Oaxaca, México.

She presented with features of social deprivation. She was unmarried, with unknown parents and four brothers who died of unknown causes.

At age 13 menstrual periods started; they occurred every 30 days and lasted 3 days. There had been no sexual activity. Her last menstruation was on April 12, 1988. At the age of 16, she noticed that her abdomen was growing gradually, and it was painful.

In 1985 she was hospitalized and nonspecific tests including an abdominal puncture were done. Results are unknown.

The patient reported that since January 1988 the large abdominal volume had made her lose her equilibrium. She had also lost the use of muscles and was fatigued by small efforts. She had had amenorrhea for 17 months, and she had edema of the lower extremities.

Physical examination. Weight on admission was 242 pounds, heart rate 88 beats/min, respiratory rate 24 breaths/min, temperature 36° C, and arterial pressure 110/70 mm Hg. She appeared older than her age with bad conformation because of the large volume of the abdomen. She was pale, with normal breasts and a small amount of pubic hair. The abdomen demonstrated collateral venous trajectories. The measurement from the pubis to the top of cyst was 128 cm. No visceral growth was found. Peristalsis was present. A pelvic examination was not done. The sphincter tonus was normal, and we could not feel either the uterus or the adnexae by rectal

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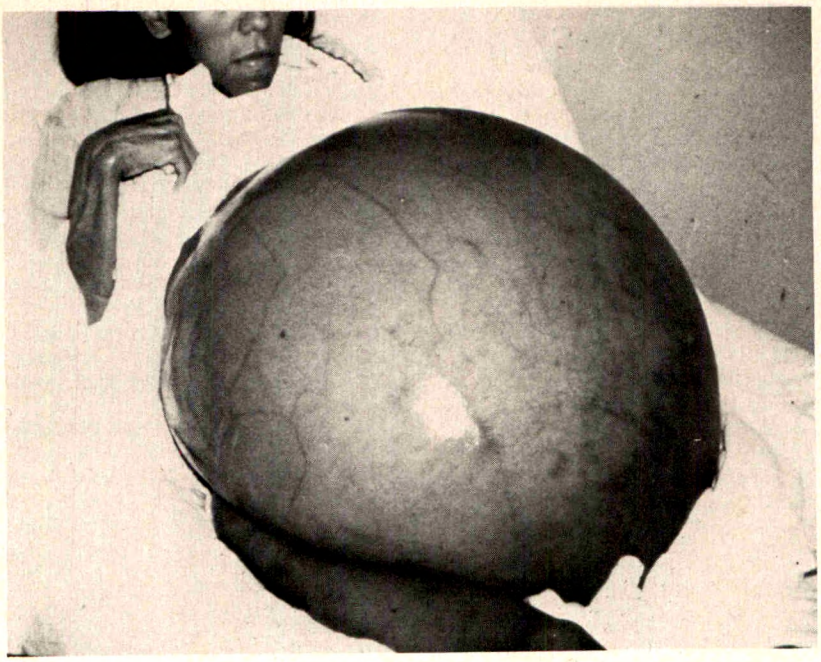


Fig. 1. Appearance of the patient on admission.

examination. The lower extremities had bimalleolar edema, loss of muscular volume, and atrophic changes of the skin (Fig. 1).

On Nov. 23, 1988, she had a cough with basal rales that made pulmonary ventilation very difficult. The cardiac rate increased to 110 beats/min. There was moderate obstruction and hypoxemia with a tendency to hyperventilation. The electrocardiogram showed a sinus tachycardia.

Renal ultrasonography showed hydronephrosis of the right kidney and moderate cholestasis in the left kidney.

The pulmonary surface was diminished on x-ray film. The right hemidiaphragm was at the level of the fourth space between the ribs and the left hemidiaphragm was at the seventh space. The cardiac shadow was horizontal.

The abdominal x-ray films and the abdominal sonogram could not be interpreted.

Treatment with digitalis and decompressive paracentesis was undertaken for 7 days with extraction of 28.8 L of fluid weighing 71.9 pounds. The fluid was negative for malignant cells.

The patient's condition improved, and we decided to perform an exploratory laparotomy on Dec. 5, 1988, with a preoperative diagnosis of a giant ovarian tumor.

Operative procedure. With the patient under general anesthesia, the abdomen was opened. A tumor of the right ovary was removed with an intraoperative diagnosis of serous cystadenoma. The rest of the structures were normal. The abdominal wall was closed with the Mayo technique. During the operation the patient received 400 ml of blood, 250 ml of plasma, and 200 ml of solutions. The postoperative course was satisfactory.

The patient's preoperative admission weight was 242 pounds (110 kg). Her postoperative weight was 88 pounds (40 kg). The weight of the tumor was 154 pounds (70 kg). After 36 days of hospitalization, her weight was 105.6 pounds (48 kg).

Comment

The patient was slowly decompressed because of the systemic effect of the tumor on the cardiovascular system. Fisher[1] reported dissemination after a puncture in the presence of a malignancy. In other reports it is suggested that the puncture is used to prevent rupture of the capsule and greater dissemination.[2]

There is little experience in cases of giant ovarian tumors. The participation of several specialties is necessary: gynecology, anesthesiology, critical care, rehabilitation, and histopathology. The more frequent complications mentioned in the literature are bleeding, supine hypotension, anesthesia complications, and splenic shock.

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The blood pressure response to infusions of angiotensin II during normal pregnancy: Relation to plasma angiotensin II concentration, serum progesterone level, and mean platelet volume

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OBJECTIVE: This study is a survey of the determinants of refractoriness to the pressor effects of angiotensin II during normal pregnancy.

STUDY DESIGN: In 25 normal pregnant women, the effective angiotensin II pressor dose was determined 88 times from the twenty-fifth to the thirty-second week of gestation. Immediately before the angiotensin II infusion, blood samples were collected and measured for plasma angiotensin II concentration, serum progesterone level, platelet count, and mean platelet volume.

RESULTS: The effective pressor dose had a significantly positive correlation with plasma angiotensin II concentration and serum progesterone level and a negative correlation with mean platelet volume.

CONCLUSION: The pregnancy-associated refractoriness to angiotensin II is physiologically determined, at least in part, by the elevated circulating levels of endogenously produced angiotensin II and by the progesterone produced by the placenta, whereas platelet activation attenuates the relative refractoriness during normal pregnancy. (Am J Obstet Gynecol 1992;166:1249-53.)

Key words: Effective pressor dose, angiotensin II, progesterone, platelets, normal pregnancy

Pregnant women are relatively refractory to the pressor effects of angiotensin II. In contrast, women who later have the development of preeclampsia lose the normal gestational resistance to the pressor effects of angiotensin II by the twenty-sixth week of gestation.[1] Thus far, volume deficit, plasma angiotensin II level, 5α-pregnane-3,20-dione, and thromboxane A_2 synthetase inhibitors are known to affect the pregnancy-associated refractoriness to the pressor effects of angiotensin II.[2-4] In normal pregnancy, the increased thromboxane biosynthesis is mainly platelet derived and is consistent with increased platelet activation.[5] Young platelets are large, and they become progressively smaller with age.[6] This condition requires much research, particularly with regard to the mean platelet volume and the pressor effect of angiotensin II.

We simultaneously determined the effective angiotensin II pressor dose, plasma angiotensin II concentration, serum progesterone level, platelet count, and mean platelet volume to survey the determinants of refractoriness to the pressor effects of angiotensin II during normal pregnancy and the interrelationships between the effective pressor dose and these parameters.

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Material and methods

Twenty-five pregnant women with single fetuses were studied. These women were managed at Kumamoto University Hospital. Full and informed written consent was obtained from the subjects for their participation. None showed symptoms or signs of preeclampsia on the first visit to the clinic, and none had a history of renal disease or a personal or family history of hypertension. The mean age of the subjects was 29.6 years (range 23 to 42 years). There were 8 primigravid and 17 multiparous subjects. In all cases gestational age was estimated from the date of the last menstrual period, and ultrasonographic measurements of the gestational sac and the crown-rump length were used to confirm the gestational age until the twelfth week of pregnancy. These patients were seen throughout the course of pregnancy at 1- or 2-week intervals and were managed with routine monitoring according to the pro-

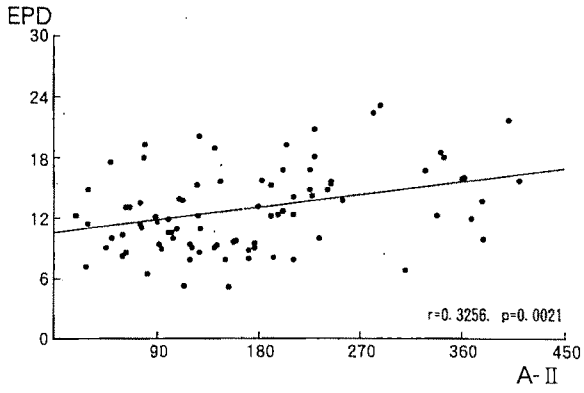


Fig. 1. Relationship between effective pressor dose and plasma angiotensin II concentrations during normal pregnancy. Effective pressor dose was significantly correlated with plasma angiotensin II concentration ($r = 0.3256$, $n = 87$, $p = 0.0021$).

tocol for the hospital, which consists of blood pressure monitoring, urinalysis for protein, body weight, and uterine fundal height measurements, and ultrasonographic measurement of fetal development. No subjects had taken aspirin, other salicylates, or nonsteroidal antiinflammatory medications for a period of 30 days before the study, and their diets were unrestricted during pregnancy.

In all studies the dose of angiotensin II required to elicit a pressor response was determined as described by Gant et al.[1] In 25 subjects the effective pressor dose was determined 88 times between the twenty-fifth and thirty-second week of gestation. The mean number of angiotensin II infusions to each patient was 3.75 ± 0.5 (range 3 to 4). The interval from one angiotensin II infusion to the next was at least 2 weeks. The patients were placed in the supine position and were tilted about 15 degrees to avoid hypotensive supine syndrome. The patients remained in this position for 20 minutes to stabilize the circulation before the angiotensin II infusion was begun. The blood pressure cuff was applied to the right upper arm. A mercury sphygmomanometer was used to measure blood pressure. The diastolic blood pressure was estimated from the fifth Korotkoff sound. After the diastolic blood pressure had stabilized, an intravenous infusion containing 1 μg of angiotensin II amide (Hypertensin Ciba, Ciba-Geigy, Ltd., Basel) per milliliter of 5% dextrose in water was given with an infusion pump (Ivac 530, Ivac Corp., San Diego). The initial dosage (<5 ng/kg of maternal weight per minute) was infused, and small progressive increments were given every 5 minutes until the diastolic blood pressure increased by 20 mm Hg over the baseline diastolic blood pressure. The minimum amount of angiotensin II amide infused per kilogram of body weight per minute that caused an increase in diastolic blood pressure of 20 mm Hg was defined as the effective pressor dose. A nonstress test was performed during each angiotensin II infusion. All patients underwent follow-up throughout pregnancy until at least 4 weeks post partum. None showed any symptoms or signs of preeclampsia.

Immediately before the angiotensin II infusion, blood samples were collected by right antecubital venipuncture for determination of the plasma angiotensin II concentration, serum progesterone levels, platelet count, and mean platelet volume. After plasma or serum was separated by centrifugation, the assay samples were stored at −20° C until they were analyzed. The plasma angiotensin II concentration and serum progesterone levels were assayed with a commercially available radioimmunoassay kit (angiotensin II kit, SMI Bristol Co., Sagamihara, Japan; DPC progesterone kit, Diagnostic Products Co., Los Angeles). All samples were assayed in duplicate. The intraassay and interassay coefficients of variation were 3.2% and 8.4% for plasma angiotensin II determinations and 6.6% and 9.3% for serum progesterone determinations, respectively. Analyses of platelet count and mean platelet volume were performed on a Coulter Counter (model S-plus II, Coulter Electronics Ltd., Luton, United Kingdom), which was an automated hematologic analyzer. Platelet counts were estimated by differential gating, mean platelet volume was measured in femtoliters.[7] Channel calibration with latex particles (platelet volume calibration latex, model No. 8.6 H; Coulter) was completed daily and later weekly to ensure sample comparability with respect to mean platelet volume and platelet distribution width values.

Data were expressed as mean ± SD. The number of samples at each time is indicated by n. Levels of significance were calculated with the unpaired Student t test. The correlation coefficient between a pair of parameters was calculated by single linear regression with a least-squares minimization equal weighting of data points. A p value <0.05 for the number of observations was considered significant.

Results

Effective pressor dose changes during pregnancy. The effective pressor doses at 25, 26, 27, 28, 29, 30, 31, and 32 weeks of gestation were 12.4 ± 2.7 ($n = 7$), 12.9 ± 3.9 ($n = 16$), 12.1 ± 3.2 ($n = 8$), 12.0 ± 4.2 ($n = 13$), 13.0 ± 3.1 ($n = 7$), 14.2 ± 5.2 ($n = 17$), 12.0 ± 3.1 ($n = 6$), and 13.1 ± 4.6 ($n = 14$) ng/kg/min, respectively. From the twenty-fifth to thirty-second week of gestation, the mean effective pressor dose ranged from 12.0 to 14.2 ng/kg/min, and the effective pressor dose was not significantly different from one gestational week to the next.

Correlations between effective pressor dose and plasma angiotensin II concentrations. The plasma angiotensin II concentrations at 25, 26, 27, 28, 29, 30, 31,

and 32 weeks of gestation were 138 ± 71 (n = 7), 124 ± 79 (n = 15), 137 ± 50 (n = 8), 195 ± 98 (n = 13), 212 ± 73 (n = 7), 178 ± 85 (n = 17), 183 ± 78 (n = 6), and 169 ± 89 (n = 14), pg/ml, respectively. The plasma angiotensin II concentration gradually increased and became stable by the twenty-ninth week of gestation. There was not a significant correlation between the plasma angiotensin II concentration and the gestational age (r = 0.1761, n = 87, p = 0.1027). In 25 normal pregnant women, however, a significant positive correlation was found between the effective pressor dose and the plasma angiotensin II concentrations (Fig. 1).

Correlations between effective pressor dose and serum progesterone level. The serum progesterone levels at 25, 26, 27, 28, 29, 30, 31, and 32 weeks of gestation were 63.6 ± 14.6 (n = 7), 63.8 ± 15.6 (n = 13), 67.6 ± 19.0 (n = 8), 67.8 ± 15.5 (n = 13), 70.1 ± 13.8 (n = 7), 92.4 ± 36.0 (n = 17), 106.8 ± 24.8 (n = 6), and 105.1 ± 38.2 (n = 15) ng/ml, respectively. The serum progesterone level gradually increased between the twenty-fifth and thirty-second week of gestation. There was a significant correlation between serum progesterone level and gestational age (r = 0.4774, n = 86, $p < 0.0001$). In 25 normal pregnant women a significant positive correlation was found between effective pressor dose and serum progesterone levels (Fig. 2).

Correlations between effective pressor dose and platelets. The platelet counts at 25, 26, 27, 28, 29, 30, 31, and 32 weeks of gestation were 215 ± 23 (n = 5), 228 ± 56 (n = 15), 224 ± 33 (n = 8), 237 ± 59 (n = 13), 229 ± 26 (n = 6), 246 ± 55 (n = 14), 219 ± 33 (n = 6), and 223 ± 53 (n = 12) $\times 10^3$/ml, respectively. The mean platelet volumes at the same weeks of gestation were 8.9 ± 0.7 (n = 5), 8.8 ± 1.0 (n = 16), 8.9 ± 1.0 (n = 8), 9.1 ± 0.8 (n = 13), 8.5 ± 0.7 (n = 6), 9.0 ± 0.9 (n = 14), 8.9 ± 1.3 (n = 6), and 9.4 ± 1.0 (n = 12) fl, respectively. In 25 normal pregnant women a significant negative correlation was found between the effective pressor dose and the mean platelet volume (Fig. 3), and a significant positive correlation was found between the effective pressor dose and platelet counts (r = 0.2405, n = 78, p = 0.0339). There was a marked negative correlation between the platelet counts and the mean platelet volume (r = −0.4578, n = 79, $p < 0.0001$).

Comment

Normal pregnant women are characterized by a relative refractoriness to angiotensin II.[8] In the study of Abdul-Karim and Assali[8] primigravid women, 21% of whom were white and 79% of whom were black, were studied sequentially from as early in gestation as possible throughout pregnancy. At early stages of pregnancy the mean effective pressor dose was 8.6 ± 0.8 ng/kg/min; this increased until the twenty-eighth week of gestation. From the twenty-sixth week until approximately the thirty-second week of gestation, the effective pressor dose remained markedly increased; the mean effective pressor dose ranged between 13.5 and 14.9 ng/kg/min.[1] In the current study the mean effective pressor dose remained between 12.0 and 14.2 ng/kg/min during this same gestational period. These changes in the effective pressor dose agree with findings reported by Gant et al.,[1,2] but the effective pressor dose values in our subjects were approximately 90% of the value they obtained in African-Americans.

The vascular sensitivity to infused angiotensin II may be dependent on racial or genetic differences. One third of the population in our study comprised multiparous women, and the age distribution was rather different from that in the study of Gant et al.[1,2] We previously reported that calcium supplementation could reduce the vascular sensitivity to infused angiotensin II.[9] Nutritional assessments of daily diet during

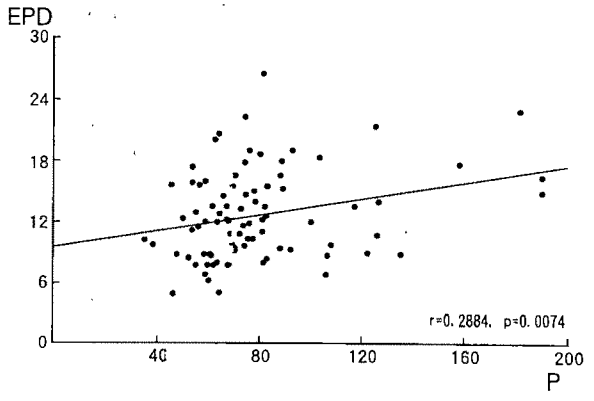


Fig. 2. Relationship between effective pressor dose and serum progesterone levels during normal pregnancy. Effective pressor dose was significantly correlated with serum progesterone level (r = 0.2884, n = 85, p = 0.0074).

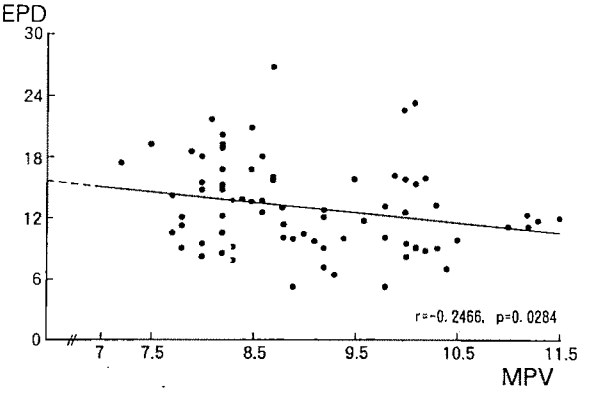


Fig. 3. Relationship between effective pressor dose and mean platelet volume during normal pregnancy. Effective pressor dose was significantly correlated with mean platelet volume (r = −0.2466, n = 79, p = 0.0284).

pregnancy revealed that pregnant Japanese women consume approximately 880 mg of calcium; this is a relatively low calcium intake. The low calcium intake during pregnancy may lead to the disparity noted between Japanese and African-Americans. With respect to the correlation between the effective pressor dose and other parameters of pregnancy, it is important to note that the effective pressor dose does not correlate significantly with gestational age. Maternal volume expansion, which can attenuate the pregnancy-associated refractoriness to angiontensin II has reached 40% in singleton pregnancies and is maintained at this level between the seventh and ninth months of pregnancy.[10] If the effective pressor dose value or blood volume expansion correlated significantly with gestational age, the evaluation of interrelationships between the effective pressor dose and other parameters that increase parallel to gestational age would have less significance because its statistical correlation is believed to be coincidental.

The renin-angiotensin-aldosterone system is remarkably altered in pregnant women. In normotensive pregnant women there are marked increases in the concentrations of plasma renin, renin activity, renin substrate, angiotensin II, and aldosterone. Angiotensin I is converted to the biologically active octapeptide angiotensin II by the converting enzyme. The conflict among studies of plasma angiotensin II concentration and our data in normal pregnancy is primarily a result of the heterogeneity of the study populations.[11] The major effect of angiotensin II is on peripheral vessel resistance. Angiotensin II depolarizes vascular smooth muscle and also has a contractile effect on vascular smooth muscle. There are two types of angiotensin II receptors in rat mesenteric artery, and their number is regulated by the circulating levels of angiotensin II. Prolonged exposure such as that which occurs in normal pregnancy, results in a time-dependent decrease in the binding capacity of the receptor with receptor degradation. Clark[12] reported that elevation of circulating angiotensin II levels selectively depresses the pressor response to exogenously administered angiotensin II and that the observed decrease in responsiveness to angiotensin II is not a result of a general hemodynamic mechanism but rather is specific for angiotensin II. A short-term blockade of endogenous angiotensin II formation by angiotensin-converting enzyme inhibitors produced a specific loss of pregnancy-associated refractoriness to angiotensin II.[13] Therefore the mean plasma angiotensin II concentration varies among individuals and with gestational age, and the interrelationships between the plasma angiotensin II concentrations and the effective pressor dose exhibited a weak (although significant) positive correlation. Thus endogenous angiotensin II may be one of the possible determinants of pregnancy-associated refractoriness to angiotensin II.

In preliminary studies Everett et al.[3] observed that the loss of refractoriness to the pressor effects of angiotensin II after delivery could be delayed by intramuscular administration of large amounts of progesterone to the mother during the later stages of labor, although intravenous administration of progesterone did not restore angiotensin II refractoriness. In an earlier study we found that the decrease in the pressor effects of angiotensin II in pregnant rats is mediated mainly by progesterone rather than by estrogen.[14] In this study a significant positive correlation was found between the effective pressor dose and the serum progesterone levels. The data obtained in the current study confirm those of our previous study. Interestingly, administration of 5α-pregnane-3,20-dione was associated with a blunting of the pressor response in late pregnancy.[3] The plasma concentrations of 5α-pregnane-3,20-dione are strikingly elevated during pregnancy, and this progestin is derived from A-ring reduction of progesterone in the liver. Progesterone decreases the concentration of angiotensin II receptors in rat uterine smooth muscle,[15] and it or a metabolite might have the same effect on vascular receptors in humans. Progesterone, in addition to endogenous angiotensin II, may be considered a possible determinant of the pregnancy-associated refractoriness to angiotensin II.

Pregnancy is accompanied by significant alterations in the hemostatic mechanism and its parameters. When subjects with normal pregnancy were compared with nonpregnant controls. O'Brien et al.[16] demonstrated a significantly lower platelet count and an increase in circulating platelet aggregation and in vitro hypoaggregability in the pregnant women. These findings suggest that normal pregnancy is associated with platelet activation, which leads to their in vivo clumping and aggregation. In this study neither the platelet count nor the mean platelet volume correlated significantly with gestational age. There was a striking negative correlation between the platelet count and the mean platelet volume. Young platelets are large, and they become progressively smaller with age.[6] In diseases characterized by a hyperdestruction and decreased life span of platelets, the platelet count decreases and the platelet volume increases.[17] The increase in mean platelet volume has been interpreted as evidence of increased platelet consumption and indicates shortened platelet life. From these findings it may be possible to show increased platelet consumption in normal pregnancy by demonstrating an increase in mean platelet volume even though the platelet count remains within the normal range. In this study a significant positive correlation was found between the effective pressor dose and the platelet count, and a significant negative correlation was

found between the effective pressor dose and the mean platelet volume. Intracellular platelet organelles are rich sources of β-thromboglobulin and 5-hydroxytryptamine. Arachidonic acid can be converted to thromboxane A_2 in platelets. Both thromboxane B_2 and 5-hydroxytryptamine are potent vasoconstrictors. The concentration of these compounds increases as a result of the release phenomenon after the activation of these platelets. Sakamoto,[18] who compared normal pregnant women with nonpregnant women, reported that β-thromboglobulin and a thromboxane A_2 metabolite (thromboxane B_2) increase significantly in plasma after the twenty-eighth week of pregnancy. Wilcox and Welch[4] reported that thromboxane A_2 synthetase inhibitor given for 3 days in anesthetized rats reduced basal thromboxane B_2 excretion by 75% and also attenuated the angiotensin II–induced rise in mean arterial blood pressure. These findings indicate that when such compounds are released from platelets, they may in part potentiate the pressor effects of angiotensin II that are associated with normal pregnancy.

In conclusion, the results of this study suggest that the pregnancy-associated refractoriness to angiotensin II is physiologically determined, at least in part, by the elevated circulating levels of endogenously produced angiotensin II and by the progesterone produced by the placenta, whereas the activation of platelets during normal pregnancy leads to attenuation of the relative refractoriness to angiotensin II. This observation is supported by the possibility that the impaired production of angiotensin II and progesterone during pregnancy and the increased destruction of platelets may lead to the loss of relative refractoriness to angiotensin II during pregnancy, such as that seen in pregnancy-induced hypertension.

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A comprehensive program for cervical cancer detection and management

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OBJECTIVE: The purpose of this study was to document some of the results of a comprehensive provincial cytology and colposcopy program for the year 1988 and also to review the impact on the incidence and mortality rates for a clinical carcinoma of the cervix.
STUDY DESIGN: This study is a retrospective analysis of the cytologic results of all patients examined provincially in 1988 and a review of the clinical records of patients diagnosed with invasive cancer and those who died of disease.
RESULTS: In 1988 490,985 women (40% of all women over the age of 15 in the population) were screened, with 9.2% showing abnormal cells. A total of 79% of women screened were <50 years old and accounted for 86.3% of all abnormal smears. Women <35 years old were more likely than older women to have moderate dyskaryosis or worse.
CONCLUSION: Intensive comprehensive cytology and colposcopy programs reduce not only the incidence and mortality of clinical carcinoma of the cervix but also rates of in situ disease and other precursors. (AM J OBSTET GYNECOL 1992;166:1254-9.)

Key words: Cytologic screening, colposcopy, squamous carcinoma of the cervix

Regions or countries that embarked on comprehensive population-based cervical cancer screening programs in the past are now enjoying the benefits of these efforts as evidenced by a significant decrease in both the incidence and mortality rates for clinically invasive squamous cell carcinoma of the cervix.[1-6] In those areas where cytologic screening has not been as intense, clinical carcinoma of the cervix continues to be one of the most common forms of malignancy in women and a not infrequent cause of death. In British Columbia,[7] a cervical cancer screening program was introduced in 1949, which had as its main purpose the identification of the early stages in the natural history of the disease that was called preclinical carcinoma. Specifically, efforts were directed toward the detection and management of carcinoma in situ and microinvasive and occult invasive carcinoma. Cytologic smears suggesting earlier phases in the disease process (mild, moderate, and severe dysplasia) had cytologic follow-up. To achieve these aims, a centralized cytology laboratory was established to provide cytologic services for the entire population of the province.

Previous reports[8-12] have documented some of the activities and results of these programs. In the late 1960s and early 1970s, the disease rate for preclinical carcinoma in reproductive age women started to increase; this coincided with the establishment of the regional colposcopy clinic service to complement the existing cytology program.[13-15] The colposcopy clinics are hospital-based and staffed by community gynecologists. All information, results, and recommendations of the cytologic and colposcopic examinations for every patient are maintained in the central cytology registry.

The purpose of this report is to document some of the results of these programs for the year 1988 and to review the impact of these programs on the incidence and mortality rates for clinical carcinoma of the cervix and the trends that have been recorded for preinvasive neoplasia.

Table I. Cytologic screening in British Columbia, 1988

Smear category	*No.*	*%*
Negative	437,658	89.1
Atypical		
Squamous	45,333	9.3
Glandular	2,368	0.5
Unsatisfactory	5,576	1.1
TOTAL	490,935	100.0

Material and methods

A central cytology laboratory in British Columbia handles all cervical cytologic screening for the province, which currently has a population of almost 3,000,000

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Table II. British Columbia screening program, 1988: Abnormal smears (squamous) by age group, first screen and previously screened

Age group	All women screened		First screen		Abnormal smears				Rate/1000 women screened	
					All women		First screen			
	No.	%	No.	%	No.	%	No.	%	First screen	Previous screen
<20	26,438	5%	13,727	52%	2,864	10.8%	955	33.3%	65.5	150.2
20-34	221,076	45%	24,131	11%	23,787	10.8%	1,880	7.9%	77.9	111.2
35-49	141,652	29%	8,053	6%	12,504	8.8%	667	5.3%	82.8	88.6
50-64	69,587	14%	4,761	7%	4,429	6.4%	202	4.6%	42.4	65.2
≥65	32,232	7%	3,547	11%	1,799	5.6%	158	8.8%	44.5	57.2
TOTAL	490,985	100%	54,219	11%	45,383	9.2%	3,862	8.5%	71.2	95.0

people. Approximately 2800 cervical cytologic smears per day are examined, and the records and results of each patient are incorporated into the computerized file in the central registry. This file now contains records on 1.5 million women. The file was used to produce cytologic data for all women who had a cervical smear in the calendar year January to December 1988. An analysis of these results by cytologic diagnosis and age group was then performed. The central file also contains information and results of all colposcopic examinations performed in the regional clinics. Data relating to all of the colposcopic examinations performed in 1988 were similarly abstracted and analyzed.

Results

During the year 1988, 490,985 women (40% of all women >15 years old in the population) had a cervical cytologic examination (Table I). Of these smears, 45,383 (9.2%) were reported as showing abnormal squamous cells. Squamous lesions consistent with moderate dysplasia or higher were present in 11,368 women. A total of 5576 smears were recorded as being unsatisfactory for interpretation.

Table II shows the number of women screened by age group, those who had their first smear performed in 1988, and the number and rates per 1000 women screened who had abnormal smears showing squamous atypia. The decision as to who enters the screening program and the frequency with which smears are done is made by the woman and her physician. The number of women screened in these various age groups reflects these decisions rather than the composition of the population. Of the women screened, 79% were under the age of 50 years; this group accounted for 86.3% of all abnormal smears during that year. Eleven percent of all women screened in 1988 had their first smear in that year and were responsible for 3862 (8.5%) of all abnormal smears. The proportion of first-time screened women was highest in those under the age of 20 years (52%), followed by women aged 20 to 34 years (11%), and those ≥65 years (11%). Age-specific rates for abnormal smears in women screened either for the first time or previously are also presented in Table II. In women previously screened, the rate decreased with advancing age. These trends are not entirely unexpected as if sufficient numbers of women in the younger age groups of a population are screened the rates of disease in those cohorts should fall over time.

Table III. Analysis of squamous atypia

Category	No.	%
Benign atypia	10,308	22.7
Mild dyskaryosis	23,707	52.2
Moderate dyskaryosis	8,399	18.5
Marked dyskaryosis	1,950	4.3
Suspicious	950	2.1
Malignant	69	0.2
TOTAL	45,383	100.0

Table III presents an analysis by cytologic abnormality for those 45,383 women found to have some form of squamous atypia on the Papanicolaou test. Approximately 75% of these individuals had cell changes consistent with benign atypia or mild dysplasia. This reflects the nature of the population screened, the majority having had several previous Papanicolaou smears. In a highly screened population, reexamination tends to result in lesions being diagnosed and managed at an earlier stage.

Table IV presents the percentage of the various cytologic abnormalities noted within each age group. Women <35 years old were twice as likely to have significant lesions (moderate dysplasia or higher) than were older women (31.5% vs 15.9%).

Table V presents each cytologic diagnosis broken down into the number and percentage of women in each category by age group. Women in the 20- to 34-year age group had the highest proportion of abnormal smears in each cytologic category, followed by women in the 35- to 49-year age group.

Table IV. Analysis of age group by degree of cytologic abnormality

Age group (yr)	Total abnormal (No.)	Cytologic diagnosis				
		Benign atypia (%)	Dyskaryosis			Suspicious/malignant (%)
			Mild (%)	Moderate (%)	Marked (%)	
<20	2,864	14	54	29	3	1
20-34	23,787	17	51	23	6	3
35-49	12,504	29	53	12	3	2
50-64	4,429	34	55	8	2	2
≥65	1,799	37	49	8	3	3
TOTAL NO.	45,383	10,308	23,707	8,399	1,950	1,019

Table V. Analysis of degree of cytologic abnormality by age group

Cytologic diagnosis	Total No. of smears	Age group (yr)									
		<20		20-34		35-49		50-64		≥65	
		No.	%	No.	%	No.	%	No.	%	No.	%
Benign atypia	10,308	401	3.9	4,130	40.0	3,624	35.2	1,488	14.4	665	6.5
Mild dyskaryosis	23,707	1,536	6.5	12,199	51.5	6,671	28.0	2,422	10.2	879	3.7
Moderate dyskaryosis	8,399	820	9.8	5,522	65.5	1,547	18.4	362	4.0	148	1.8
Marked dyskaryosis	1,950	86	4.4	1,334	68.4	387	19.8	84	4.3	59	3.0
Suspicious/malignant	1,019	21	2.0	602	59.0	275	27.0	73	7.2	48	4.7
TOTAL NO.	45,383	2,864		23,787		12,504		4,429		1,799	
Percentage screened		5		45		29		14		7	

During the period of the study, it was our policy to recommend colposcopic evaluation for all patients with marked dyskaryosis or higher on Papanicolaou smear. Colposcopic examination was also recommended in patients with two or more consecutive moderately dyskaryotic smears and in cases where mild dyskaryosis was noted over a 24-month period.

In 1988 the 22 regional colposcopic clinics recorded 12,426 patient visits for diagnosis, treatment, and follow-up. Of these, 5667 women were attending a colposcopy clinic for the first time. In addition, a further 3234 women returned for repeat examination. Abnormal cytologic smears were responsible for 85.1% of all new patient colposcopy visits. Table VI lists the presenting cytologic abnormality for those women who had a first colposcopic examination in 1988. Of these women 72.7% were referred because of moderate or higher dyskaryosis on cytologic smears. A total of 3326 (74.2%) of new patients referred because of cytologic abnormality were judged to have satisfactory colposcopic examination on the basis of visualization of both the entire squamocolumnar junction and the lesion margins.

The age distribution of those women having a colposcopic examination for the first time in 1988 is presented in Table VII; 68% were <35 years of age and 92.8% were ≤50 years of age. As might be expected, the rate of satisfactory examinations correlated with the ages of the patients, with young patients having a much higher rate of satisfactory examinations.

The histologic diagnoses of all patients seen in 1988 who had colposcopically directed biopsies are presented in Table VIII. Twenty-eight patients had invasive carcinoma.

Fig. 1 presents the changes in the incidence and mortality rates for clinical invasive squamous carcinoma of the cervix in British Columbia from 1955 to 1988. The rates have shown a gradual decrease from an initial incidence rate of 28.4 per 100,000 women and a mortality rate of 11.4 to the current figures of 4.2, a reduction of 85%, and 2.3, a reduction of 80%, respectively. In addition, the rate and number of in situ carcinomas diagnosed in the screened population have decreased in later years, particularly in the past decade in the younger age group.

Fig. 2 presents the number of cases of in situ squamous carcinoma diagnosed per year between 1955 and 1988. The increase in the first 20 years is due to enhanced efforts to expand the program, resulting in detection of disease in previously unscreened women, prevalence cases, and, to a much lesser extent, to the increase in the population. The marked increase be-

Table VI. Results of colposcopic examination in new patients, 1988: Cytologic diagnosis

	Benign atypia	*Dyskaryosis*			*Suspicious*	*Malignant*	*Total*
		Mild	*Moderate*	*Marked*			
No.	150	1028	1255	1255	568	53	4309
%	3.5	23.9	29.1	29.1	13.2	1.2	100

tween 1974 and 1979 corresponded to the time that the colposcopy program was established and reflects a marked increase in the incidence rate of in situ carcinoma in our population. The cytology screening program predicted 2200 cases of carcinoma in situ in 1979.

The rates of carcinoma in situ per 1000 women screened by age group for the past 9 years in 3-year intervals are presented in Fig. 3. Although the rate in women >40 years old has remained constant, younger women, particularly those in the 25- to 29-year age group, have shown a marked decrease. These trends have occurred because of the colposcopy program, which, over time, has resulted in more of the earlier lesions being diagnosed and treated.

In 1988 the average cost per smear was $6.80. This includes all laboratory costs and the salaries of medical, technical, and clerical staff. Physician office fees are not included in this amount. The colposcopy service in the regional clinics is funded on a sessional basis. A session is approximately 3½ hours in length, during which an average of 12 patients are seen. The actual number of patients seen per session in the various clinics ranged from 8 to 35 and was related to the size of the community served, frequency of clinics held, and number of support personnel available for the session. If all patients with mild dyskaryosis or higher grades of abnormality were to have colposcopy, as suggested by some authorities, a fourfold increase in sessional funding would be required. All medical and hospital costs are covered by universal government health insurance plans.

Comment

Preclinical cervical carcinoma is a condition that is well suited to early detection by a screening program. Although squamous carcinoma of the cervix is a common disease, it has a long preclinical phase so that intervention at this stage can influence the natural history of the disease to prevent progression from a preinvasive to an invasive carcinoma in the majority of instances.

Although much has been written and a great deal of knowledge accumulated with regard to the causes and epidemiologic features[16-21] of squamous neoplasia of the cervix, primary prevention to date has not been particularly effective. Most efforts with regard to the control of cervical cancer have been directed toward secondary prevention with cytologic smears in a screening program. The detection of early preclinical invasive cancer, known as microinvasive or occult invasive carcinoma, in addition to the detection of intraepithelial forms of the disease, is also desirable, as in general the earlier lesions tend to have a better prognosis than those with more advanced stages of disease.

The goal of reducing the incidence and mortality from squamous carcinoma of the cervix can be achieved

Table VII. Age distribution of new patients by category of colposcopic examination

Age group (yr)	*Examination category*		*Total*
	Satisfactory	*Unsatisfactory*	
<20	312 (89.7%)	36	348
20-24	1020 (87.0%)	152	1172
25-29	974 (81.0%)	228	1202
30-34	619 (73.4%)	224	843
35-39	436 (68.3%)	202	638
40-44	262 (62.2%)	159	421
45-49	129 (51.0%)	124 (49.0%)	253
50-54	63	88 (58.3%)	151
55-59	41	55 (57.3%)	96
60-64	14	43 (75.4%)	57
65-69	14	31 (68.9%)	45
≥70	8	19 (70.4%)	27
TOTAL	3892	1361	5253

Table VIII. Colposcopic biopsy diagnosis for new patients, 1988

Histologic diagnosis	*Examination category*		*Total*
	Satisfactory	*Unsatisfactory*	
Negative	405	239	644
Benign atypia	462	133	595
Condyloma	73	15	88
Mild dysplasia	755	226	981
Moderate dysplasia	656	193	849
Severe dysplasia	732	216	948
Carcinoma in situ	395	181	576
Microinvasive	6	6	12
Occult invasive	5	4	9
Clinically invasive	4	3	7
TOTAL	3493	1216	4709

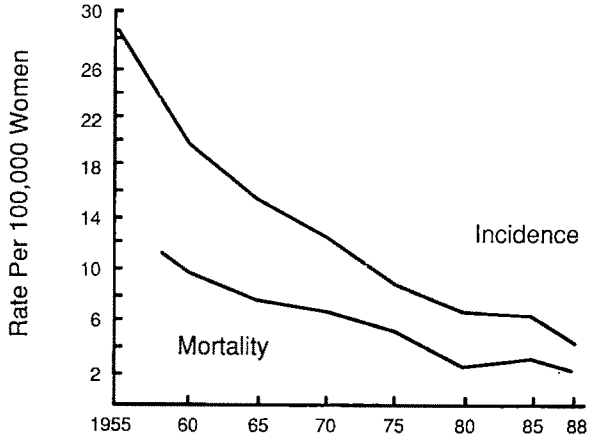


Fig. 1. Incidence and mortality rate in women >20 years old, British Columbia, 1955 to 1988.

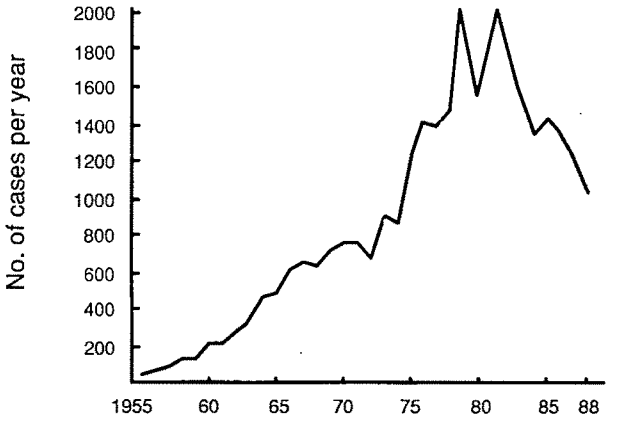


Fig. 2. Cases of carcinoma in situ detected in women ≥20 years old in British Columbia, 1955 to 1988.

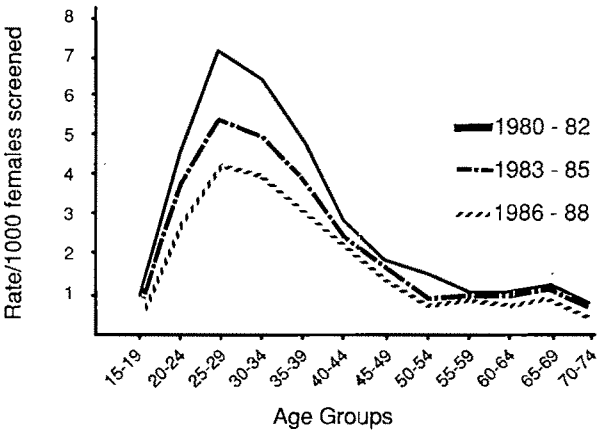


Fig. 3. Carcinoma in situ rates in British Columbia.

through screening programs, as described in this and other reports. The key element in cervical control programs has been, and continues to be, cytologic screening. In areas where facilities and resources for diagnosis and treatment are limited, emphasis should be on increasing the screening net to encompass as many women at risk as possible, particularly those who have never been screened, rather than on increasing the frequency of smears within a given population.

The data presented in this report are a measure of the levels of activity that have occurred in British Columbia to achieve significant results. It should be noted that most of the initial decrease in incidence and mortality occurred before the introduction of the colposcopy component of our program. There is no doubt, however, that before colposcopy, in many women who were managed by the traditional method of cone biopsy for assessment followed by hysterectomy, the disease was overtreated. Colposcopy represents an advance that developed as a response to the problem of increasing disease rates noted in younger women in the early 1970s. From the data presented, it is apparent that only a small proportion of all women (approximately 10%) within a screened population such as ours will have an abnormality on the Papanicolaou test. Of these, approximately 75% of smears showing squamous atypia will be in the low-grade category of mild dyskaryosis or benign atypia. It is apparent that if resources are needed to evaluate and assess colposcopically all women seen initially with these mild degrees of change, then the cost will be considerable. Furthermore, if the practice is then to treat these early, low-grade lesions, the cost escalates dramatically. Many studies[22-25] have shown that, in particular, the milder grades of atypia may regress spontaneously without specific treatment.

Reports[26-28] have appeared advocating the use of human papillomavirus deoxyribonucleic acid testing with cervical cytology and colposcopy in an attempt to better identify and characterize precursor lesions. These tests, however, involve additional expense and do not represent a substitute for the traditional method of cytologic screening.

Efforts directed toward the development of effective automated screening systems continue and offer the promise of reducing the cost of cytologic screening and increasing the reliability of the test. However, until better methods are evident and affordable, every effort should be made to continue with existing technology and expanding the use of cytologic screening programs. Areas with established programs should continue to make the program as comprehensive as possible and work toward improving quality control in their cytology and colposcopy programs.

The use of colposcopy as an adjunct to cervical cancer

detection and treatment programs is particularly helpful but needs to be used appropriately and with expertise to optimize its potential.

The aim of further reducing death rates from cervical cancer is achievable with current knowledge and technology and represents a continuing challenge for all involved in these programs.

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Factors affecting the dose response to oxytocin for labor stimulation

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For nearly 40 years synthetic oxytocin has been used for labor stimulation by titrating dosage rate to uterine contractions. We used a computerized data base to determine variables affecting the dose response to oxytocin in 1773 pregnancies. Statistically important predictors of required oxytocin dosage included cervical dilatation, parity, and gestational age. Maternal body surface area was found to be associated with a higher oxytocin dosage in women undergoing induction of labor. However, the broad range of the statistical confidence intervals precluded prediction of a given pregnancy's oxytocin requirement. (Am J Obstet Gynecol 1992;166:1260-1.)

Key words: Oxytocin, labor augmentation, labor induction

Labor stimulation with oxytocin is a very common practice in modern obstetrics. The contemporaneous method of administering oxytocin is essentially an individualized bioassay wherein dosage is titrated to uterine contractions. Typically, oxytocin is infused beginning with a low dose and thereafter slowly increasing the infusion rate until adequate contractions are achieved.[1] This method is applied regardless of pregnancy factors that could conceivably influence dose-response characteristics for oxytocin. For example, it seems that an obese woman should require a higher oxytocin dosage than a petite woman with similar pregnancy characteristics. Although many clinicians suspect that several pregnancy factors may influence the response to oxytocin, these potential oxytocin dose-response factors have not been investigated.

The purpose of this study was to determine if factors such as maternal size, duration of pregnancy, and cervical dilatation influenced the oxytocin dosage required for labor stimulation. If such factors were identifiable, the resultant ability to adjust oxytocin dosage regimens a priori could potentially improve labor stimulation efficiency.

Material and methods

This study involving 1773 pregnancies was performed at Parkland Memorial Hospital. Between Nov. 1, 1990, and May 31, 1991, oxytocin was administered by nurses trained in labor stimulation according to a written protocol. The oxytocin regimen described was part of another ongoing investigation. Briefly, the oxytocin regimen used consisted of a starting dose of 6 mU/min with incremental increases of 6 mU/min at 20-minute intervals with a maximum permitted infusion rate of 42 mU/min. Half-dose increments were used in the presence of uterine hyperstimulation. The infusate consisted of 20 U of synthetic oxytocin (United States Pharmacopeia) diluted in 1000 ml lactated Ringer's solution. Electronic fetal monitoring was required, and physicians were in attendance in the labor unit. Oxytocin dosages were regulated to achieve a minimum of three contractions per 10 minutes while avoiding uterine hyperstimulation, which was defined as uterine contractions of ≥2 minutes duration or six or more contractions per 10 minutes. Induction of labor was carried out in pregnancies requiring intervention for maternal or fetal complications without spontaneous labor or ruptured membranes. Cases classified as having augmentation of labor included those pregnancies with ineffective spontaneous labor or rupture of membranes without labor.

Nurses attended each delivery and completed a perinatal data sheet. Research nurses assessed the data for consistency and completeness before electronic storage. Moreover, the labor stimulation record, which contained oxytocin infusion rate, cervical examination, fetal heart rate, contraction quality, and labor complications, was abstracted by the investigators and computerized.

Stepwise multiple regression analysis was performed for pregnancy factors that may be associated with the maximum oxytocin dosage required for labor stimulation. These factors included maternal age, race, parity, cervical dilatation, and body surface area; delivery route; use of epidural analgesia; presence of chorioam-

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6/1/35280

Table I. Multiple regression relationship of material, neonatal, and labor demographics to the maximum dosage of oxytocin required for labor stimulation

	Labor augmentation (n = 1011)			*Labor induction (n = 762)*		
	Mean ± SEM	*No.*	*p**	*Mean ± SEM*	*No.*	*p**
Maternal factors						
Age (yr)	22.9 ± 0.12	—	NS	22.8 ± 0.15	—	NS
Race			NS			NS
Black	—	370 (36%)	—	—	229 (30%)	
Hispanic	—	445 (44%)	—	—	347 (46%)	
White	—	186 (18%)	—	—	171 (23%)	
Nulliparous	—	606 (60%)	0.0001	—	408 (54%)	0.0210
Cervix at start of oxytocin	3.5 ± 0.04	—	0.0001	1.7 ± 0.03	—	0.0001
Body surface area (m^2)	1.8 ± 0.01	—	NS	1.8 ± 0.01	—	0.0004
Infant factors						
Birth weight (gm)	3265 ± 12	—	NS	3207 ± 18	—	NS
Gestation age (wk)	39.0 ± 0.05	—	0.0624	39.6 ± 0.08	—	0.0018
Labor factors						
Vaginal delivery	—	874 (86%)	NS	—	611 (80%)	NS
Epidural anesthesia	—	45 (4%)	NS	—	14 (2%)	NS
Chorioamnionitis	—	96 (9%)	NS	—	51 (7%)	NS

NS, Not significant.
*Student *t* test of multiple regression coefficient.

nionitis; gestational age; birth weight; and condition of newborn.

The significance level for entry of variables into the model and for remaining in the model was $p < 0.15$. Multiple regression analysis was performed on the model selected by the stepwise analysis to account for intercorrelations between variables. The selected prediction equation estimated by multiple linear regression was then used to establish 95% confidence intervals for the prediction of the maximum oxytocin dosage. The statistical analysis was performed with the SAS/STAT statistical program.[2]

Results

Of the 1773 pregnancies, 1011 (57%) received oxytocin for labor augmentation and the remaining 762 for labor induction. Results of multiple regression analysis for maternal, neonatal, and labor demographics in relationship to required maximum oxytocin dosage are summarized in Table I. The statistically important predictors of the maximum dosage of oxytocin required during both labor augmentation and induction included cervical dilatation, parity, and gestational age. Specifically, higher oxytocin dosages were required when cervical dilatation was ≤2 cm, the mother was nulliparous, and duration of pregnancy was ≤36 weeks. Maternal body surface area was found to be associated only with higher oxytocin dosage in women undergoing labor induction. These significant predictors of the infusion rate of oxytocin were then used to derive a predictive formula for the maximum rate of oxytocin infusion. For example, the formula predicted that a term, nulliparous woman with a cervix dilated 2 cm would require a maximum dosage of 36 mU/min for labor induction. However, the standard deviation of this prediction exceeds 20 mU/min, and the 95% confidence interval of the predicted infusion rate would range from 2 to 42 mU/min.

Comment

Our data confirm the suspicion of clinicians that cervical dilatation, gestational age, and parity influence pregnancy response to oxytocin. However, the broad range of the confidence intervals precludes prediction of a given pregnancy's oxytocin requirement. In conclusion, knowledge of cervical dilatation, gestational age, parity, or body surface area offers no practical predictive advantage in determining the dose response to oxytocin used for labor stimulation. Until the pharmacokinetics of oxytocin are better understood, each pregnant woman receiving oxytocin for labor stimulation will continue to represent an individual bioassay.

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Middle cerebral artery flow velocity waveforms in normal and small-for-gestational-age fetuses

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The middle cerebral artery flow velocity waveforms were obtained in 16 normal fetuses in a longitudinal study and in 128 normal fetuses in a cross-sectional study with pulsed Doppler ultrasonography. The pulsatility index values of the middle cerebral artery were higher at 25 to 30 weeks' gestation than those observed during the two periods of major cerebral cellular multiplication in the human fetus. In 9 of the 16 fetuses studied longitudinally we compared the pulsatility index values obtained at the end of gestation with those obtained at 1 month after delivery; no significant difference was observed. The pulsatility index of the middle cerebral artery was also determined in 33 small-for-gestational-age fetuses. A middle cerebral artery pulsatility index value below our normal range was recorded in 9 of the 33 small-for-gestational-age fetuses (27.3%). These small-for-gestational-age fetuses with abnormal pulsatility index values had a significantly higher incidence of abnormal fetal heart rate and admission into the neonatal intensive care unit. There were three deaths in the nine small-for-gestational-age fetuses with abnormal pulsatility index values (33.3%) and three deaths in the 24 small-for-gestational-age fetuses with normal pulsatility index values (12.5%). Our data indicate that the pulsatility index of the middle cerebral artery in the normal human fetus has a parabolic pattern during pregnancy and does not change significantly after delivery. The results suggest that the small-for-gestational-age fetus with a normal middle cerebral artery pulsatility index is at lower risk than the fetus with abnormal pulsatility index values; moreover, management of the small-for-gestational-age fetus may be aided by the study of the middle cerebral artery waveforms. (Am J Obstet Gynecol 1992;166:1262-70.)

Key words: Middle cerebral artery, small for gestational age, intrauterine growth retardation, pulsatility index, brain-sparing effect

Several studies of fetal cerebral artery blood flow velocity waveforms have been reported in the third trimester of pregnancy in normal, intrauterine growth retarded (IUGR),[1, 2] and anemic fetuses.[3] The studies emphasized a decrease in downstream impedance in the cerebral vasculature of IUGR fetuses attributed to a brain-sparing effect.[1] Additionally, a reduction in PO_2 established in the umbilical artery through cordocentesis at the end of gestation[4] has been associated with the decrease in pulsatility index noted in the cerebral arteries of normal fetuses during this period of gestation.[5] Normal values for the velocity waveforms of different cerebral arteries have been published by a number of investigators.[1, 2, 5] In many cases the pulsatility index values were derived from cross-sectional Doppler studies, and no longitudinal report on the cerebral blood flow velocity waveforms early in the second trimester of pregnancy (i.e., 15 weeks) has been presented.

Our study presents a serial assessment of the pulsatility index for the middle cerebral artery starting early in the second trimester. Additionally, the pulsatility index of the middle cerebral artery was assessed in 128 normal fetuses in a cross-sectional study. The data obtained in the cross-sectional study have permitted an evaluation of the variability of the pulsatility index within a given population and have provided the basis for the development of normal values of the pulsatility index for the middle cerebral artery in the human fetus. The predicted values derived from the cross-sectional study were compared with the average longitudinal curve. Furthermore, to evaluate the variability of the middle cerebral artery velocity waveforms before delivery and in the first month after delivery, the pulsatility index value of the middle cerebral artery was obtained in nine normal fetuses at the end of gestation and at 1 month post partum. Finally, middle cerebral artery velocity waveforms of 33 small-for-gestational-age (SGA) fetuses are reported and compared with those obtained in normal fetuses.

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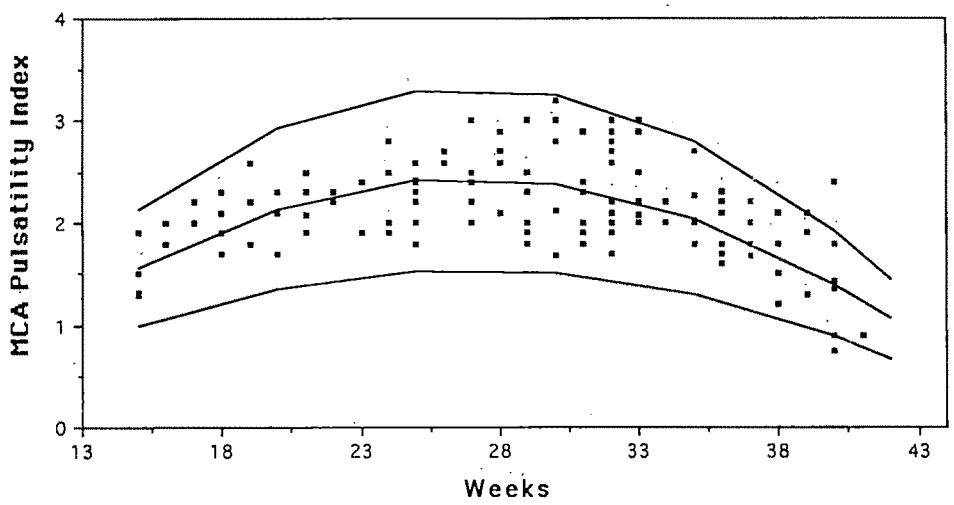


Fig. 1. Normal range of middle cerebral artery pulsatility index (*PI*) as a function of gestational age constructed from study of 128 normal fetuses. Lower limit = Predicted pulsatility index − (2 × 0.184 × Predicted pulsatility index); Upper limit = Predicted pulsatility index + (2 × 0.184 × Predicted pulsatility index).

Material and methods

This study was done with data from 16 normal fetuses (group 1), 128 normal fetuses in a cross-sectional study (group 2), and 33 SGA fetuses (group 3). Informed consent was obtained from all patients. Twenty-five subjects in our study population participated in other investigations.[6] The women in the first two groups were delivered of their fetuses after 37 weeks of gestation; comprehensive pediatric assessment revealed no evidence of growth abnormalities or congenital anomalies. Birth characteristics such as weight, length, head circumference, and abdominal circumference were within the normal ranges for singletons determined by Usher and McLean.[7] Nine normal fetuses monitored longitudinally were studied 1 month after delivery; two of them were studied four times in the first 6 months after delivery.

Fetal age determination. Fetal age in group 1 was determined from the date of the last menstrual period and confirmed by first-trimester and early second-trimester ultrasonographic examinations (crown-rump length, biparietal diameter, and femur diaphysis length measurement).[8]

Gestational age in groups 2 and 3 was determined by both menstrual history and sonographic assessment of fetal biometry from either a scan before 20 weeks' gestation or, if such a scan had not been performed, the first ultrasonographic assessment in this study. Gestational age was based on (1) the date of the last menstrual period when it differed by <2 weeks from the gestational age determined by sonographic parameters or (2) the sonographic assessment if the patient was unsure of the date of her last menstrual period or if the discrepancy between menstrual and sonographic gestational age was >2 weeks. SGA fetuses were defined on the basis of ultrasonographic measurements of head and abdominal circumferences[8] 2 or more SD below the mean and confirmed by a low birth weight.[7]

The data obtained from the Doppler ultrasonographic studies were not used in the clinical management of the patients.

Doppler ultrasonographic examinations. The normal and SGA fetuses were examined when the mothers were in a semirecumbent position. Exposure of the fetal brain to Doppler ultrasonography was kept to a minimum estimated to be around 1 to 5 minutes. Examination of the middle cerebral artery was performed as reported previously.[9] A PASS II (General Electric Medical Systems, Milwaukee, Wis.) with pulsed Doppler beam was used in conjunction with a 2.5 MHz real-time imaging device with a 2 mm sample volume. A 0 to 50 Hz pass filter was used. Ultrasonographic energy was 114 mW/cm² spatial peak temporal average intensity; although it exceeded the 100 mW/cm² level recommended by the Food and Drug Administration for fetal Doppler ultrasonography,[10] the intensity was well below the known peak acoustic field emission of 180 mW/cm² for preenactment ultrasonographic diagnostic devices. In addition, the American Institute of Ultrasound in Medicine recently reported that no independently confirmed significant biologic effects in mammalian tissues have been noted with focused ultrasonographic intensities below 1 W/cm².[11]

Doppler examinations of the middle cerebral artery in the fetuses of group 1 were carried out at 3- to 4-week intervals from 15 to 18 weeks' gestational age

Table I. Modeling of individual middle cerebral artery pulsatility index curves obtained in 16 normal fetuses monitored serially during gestation

Patient	$y = a_0 + a_1 (x) + a_2 (x)^2$	*p Value*	R^2
A	$y = -3.203 + 0.416\,x - 0.008\,x^2$	>0.05	66
B	$y = -1.053 + 0.229\,x - 0.004\,x^2$	>0.05	24
C	$y = -2.388 + 0.332\,x - 0.006\,x^2$	<0.05	93
D	$y = -2.301 + 0.344\,x - 0.007\,x^2$	<0.05	80
E	$y = -2.496 + 0.336\,x - 0.006\,x^2$	<0.05	92
F	$y = -2.173 + 0.333\,x - 0.006\,x^2$	<0.05	91
G	$y = -2.339 + 0.328\,x - 0.006\,x^2$	<0.05	93
H	$y = -2.357 + 0.339\,x - 0.006\,x^2$	<0.05	72
I	$y = -2.380 + 0.339\,x - 0.006\,x^2$	<0.05	91
J	$y = -2.397 + 0.341\,x - 0.006\,x^2$	<0.05	85
K	$y = -2.324 + 0.335\,x - 0.006\,x^2$	<0.05	81
L	$y = -2.368 + 0.333\,x - 0.006\,x^2$	<0.05	91
M	$y = -2.004 + 0.304\,x - 0.006\,x^2$	<0.05	88
N	$y = -0.881 + 0.240\,x - 0.005\,x^2$	>0.05	26
O	$y = -2.732 + 0.371\,x - 0.007\,x^2$	<0.05	86
P	$y = 1.324 + 0.259\,x - 0.005\,x^2$	<0.05	88

y, Pulsatility index; *x*, gestational age.

(mean ± SD: 16.3 ± 1.5 weeks) to 35 weeks, and at 1- to 3-week intervals thereafter until delivery. After 35 weeks we decided to study the middle cerebral artery more frequently because the time of delivery could not be predicted. The middle cerebral artery of nine fetuses in the longitudinal study was studied as seen in utero at 1 month after delivery. In two fetuses the middle cerebral artery was studied at 1, 4, 12, and 24 weeks after delivery.

The gestational age of the fetuses in group 2 at the time of the study ranged between 15 and 41 weeks (29 ± 7.2 weeks, mean ± SD). In 30 fetuses, two-dimensional directed pulsed Doppler waveforms (Acuson 128; Acuson Co., Mountain View, Calif.) were compared with color-flow Doppler imaging (Acuson 128). Before the middle cerebral artery was interrogated with pulsed Doppler ultrasonography, the color-flow mapping was successively used to confirm that the sample volume had been positioned on the middle cerebral artery and that the angle between the beam and the vessel was <20 degrees.

The gestational age of the SGA fetuses (group 2) ranged from 20 to 37 weeks (31 ± 4.3 weeks, mean ± SD) at the time of the study and from 24 to 42 weeks (33.6 ± 3.9 weeks, mean ± SD) at the time of delivery.

Data analysis. The Doppler velocity waveforms were recorded on videotape, and a digitized computer system (Digisonics Inc., Houston) calculated the following variables: peak systolic velocity, lowest diastolic velocity, and mean velocity. Three consecutive waveforms were analyzed, and the results were averaged. The pulsatility index (Peak systolic velocity − Lowest diastolic velocity)/Mean velocity)[12] was then calculated. Measurements were obtained only during fetal apnea, which was defined as the absence of visible changes in the waveform patterns for ≥15 waveforms.

The intraobserver error (coefficient of variation) for the pulsatility index of the middle cerebral artery was 5.6% as reported previously.[13]

Changes in pulsatility index with gestational age for the fetuses of group 1 were characterized quantitatively by fitting mathematic functions to the experimental data. Regression analysis was used to compare the pulsatility index with gestational age for each fetus. We looked for the optimal polynomial model on the basis of two criteria: (1) coefficients different from zero and (2) maximal coefficient of determination (R^2). Analysis of variance was used to determine whether the regression accounted for a significant part of the variability in the dependent variable. A p value <0.05 was considered to indicate statistical significance. Assessment of the variability of the pulsatility index of the middle cerebral artery during pregnancy was carried out with the measurements made on the fetuses in group 2. There was only one pulsatility index measurement per fetus in this data set. A second-degree polynomial was fitted to the data with the method of least squares and predicted values calculated at each time point where an actual measurement was made. The differences between actual and predicted pulsatility index values were determined and plotted. Because there appeared to be an increase in variability with menstrual age, these differences were expressed as a percentage of the predicted values (percent difference). The mean and SD of the percent difference were calculated, and twice the latter was used as the measure of pulsatility index variability at different times in pregnancy (Fig. 1).

A paired Student's t test was used for statistical com-

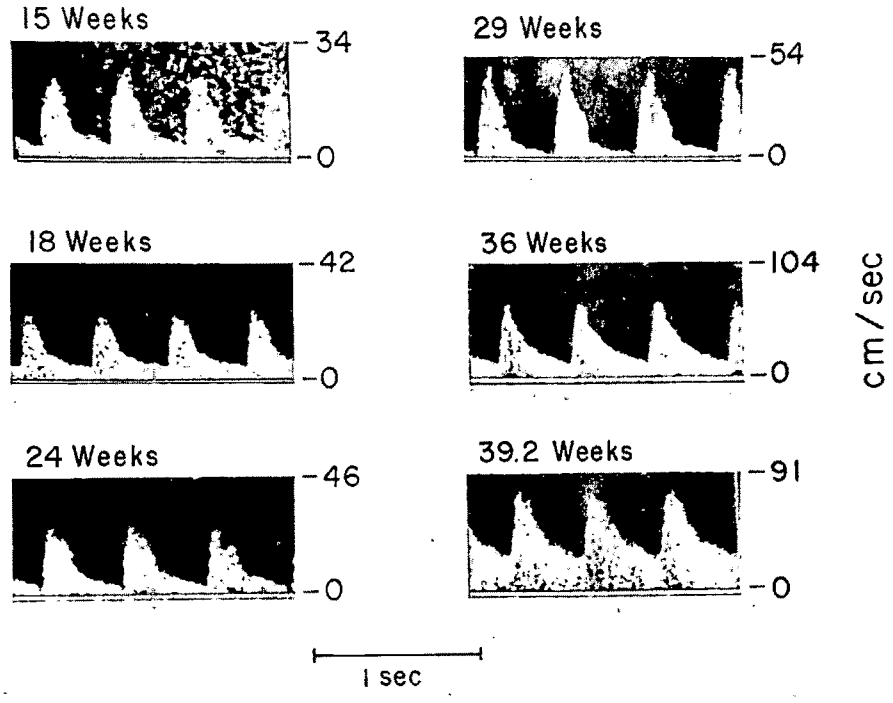


Fig. 2. Flow velocity waveforms of fetal middle cerebral artery velocity waveforms at different gestational ages.

parison of pulsatility index values in the middle cerebral artery of the fetuses and infants. A probability value of <0.05 was considered to indicate statistical significance.

The pulsatility index values for the SGA fetuses (group 3) were measured and plotted with our normal range.

In the SGA fetuses we obtained the sensitivity, specificity, positive predictive value, and negative predictive value for the middle cerebral artery pulsatility index, with the test status being above or below our normal reference range for gestational age (predicted value ± 2 SD) and the outcome status being presence or absence of adverse perinatal outcome (defined as admission to the neonatal intensive care unit for >12 hours or perinatal death).

Results

Initially the patients of group 1 were 34 normal fetuses. The data of 18 fetuses monitored longitudinally were not included in our analysis because of preterm labor ($n = 1$); first measurement after 18 weeks' gestation, an inadequate number of measurements, or both ($n = 13$); hypertension ($n = 3$); or multicystic kidney ($n = 1$). The mothers of group 1 were delivered of fetuses between 38 and 42 weeks (39.6 ± 1.1 weeks, mean ± SD). A total of 144 examinations were performed. On 16 occasions the middle cerebral artery could not be evaluated on first attempt, because of either fetal position or fetal movement. In these cases the examination was repeated within 1 week. On six occasions the measurement of the middle cerebral artery was not obtained.

In each of the 30 fetuses studied with pulsed Doppler and color flow mapping the sample volume was found over the middle cerebral artery in the color image after the middle cerebral artery was obtained with pulsed Doppler ultrasonography. The angle was always <20 degrees.

The best model in group 1 was a second-order polynomial ($y = a_0 + a_1x + a_2x^2$) (Table I). Coefficient a_0 was different from zero in nine cases; coefficient a_1 was different from zero in 13 cases; and coefficient a_2 was different from zero in 14 cases. The middle cerebral artery waveforms manifested a higher diastole at 15 to 20 weeks and at the end of gestation (Fig. 2); this was associated with a lower pulsatility index value between 15 and 20 weeks' gestation and, late in the third trimester of pregnancy, a higher value at 25 to 30 weeks' gestation. Fifteen of the 16 individual fetuses showing evidence of a parabolic change in pulsatility index with gestational age were used to specify the average longitudinal curve by determination of the mean coefficient values. The function is given in Table II.

Table II. Comparison of cross-sectional and average longitudinal curves

Gestational age (wk)	*Predicted pulsatility index* Average longitudinal curve*	*Predicted pulsatility index† Cross-sectional curve*	*Difference*	*% Difference*
15	1.308	1.568	0.260	16.58
16	1.448	1.707	0.259	15.17
17	1.576	1.834	0.258	14.06
18	1.691	1.949	0.258	13.23
19	1.794	2.051	0.257	12.53
20	1.886	2.142	0.256	11.95
21	1.964	2.220	0.256	11.53
22	2.031	2.287	0.256	11.19
23	2.085	2.341	0.256	10.93
24	2.127	2.383	0.256	10.74
25	2.157	2.413	0.256	10.60
26	2.175	2.430	0.255	10.49
27	2.180	2.436	0.256	10.50
28	2.174	2.429	0.255	10.49
29	2.155	2.411	0.256	10.61
30	2.124	2.380	0.256	10.75
31	2.080	2.337	0.257	10.99
32	2.024	2.282	0.258	11.30
33	1.957	2.214	0.257	11.60
34	1.877	2.135	0.258	12.08
35	1.784	2.043	0.259	12.67
36	1.680	1.940	0.260	13.40
37	1.563	1.824	0.261	14.30
38	1.434	1.696	0.262	15.44
39	1.293	1.556	0.263	16.90
40	1.140	1.403	0.263	18.74
41	1.028	1.175	0.147	12.51
42	0.865	0.995	0.130	13.06

PI, Pulsatility index; *GA*, gestational age.
*PI = $-2.256 + (0.3293GA) + (-0.00611GA^2)$.
†PI = $-1.9763 + (0.32737GA) + (-0.006071GA^2)$.

In nine infants the pulsatility index of the middle cerebral artery after delivery did not show any significant difference when compared with that obtained at the end of gestation (1.32 ± 0.24 vs 1.29 ± 0.19, mean ± SD) (Fig. 3). In two normal fetuses studied four times after delivery the pulsatility index did not appear to change when compared with the values obtained at the end of gestation (Fig. 4).

The polynomial function Pulsatility index = $-1.9763 + 0.32737$ (menstrual age) $- 0.0060717$ (menstrual age)^2 $R^2 = 44.8\%$ fitted to the cross-sectional data is given in Fig. 1. All coefficients were statistically significantly different from zero by t test. Reasonable agreement was obtained between this standard curve for the pulsatility index of the middle cerebral artery and values derived from the average longitudinal curve for this parameter as shown in Table II. Use of the percent deviations eliminated the change in variability with menstrual age, the mean percent difference being -0.08% (not significantly different from zero by t test) and the SD 18.4%. The standard curve for the pulsatility index of middle cerebral artery constructed from these data is given in Table III.

One SGA fetus studied at 20 weeks' gestation presented head and abdominal circumference below our reference range; the pregnancy was complicated by oligohydramnios. This fetus had reverse flow in the umbilical artery, and fetal demise was diagnosed 4 weeks later.

All fetuses estimated to be SGA in utero turned out to be SGA at birth. In 9 SGA fetuses the middle cerebral artery was below our normal reference range (Fig. 5): 2 of these died in utero; 1 died soon after birth; 5 had fetal distress or were admitted to the neonatal intensive care unit; and 1 was normal. Two of the fetuses transferred to the neonatal intensive care unit for further evaluation had a good short-term outcome and were transferred to the normal nursery within 12 hours. In two fetuses who died in utero the time between the study of the middle cerebral artery and fetal demise ranged from 2 to 4 weeks. The SGA fetuses with a normal value of pulsatility index ($n = 24$) had no complications ($n = 17$); 1 fetus died in utero, 2 fetuses died after delivery, and 4 others had fetal distress or were transferred to the neonatal intensive care unit. The two fetuses with normal middle cerebral artery pulsatility indexes who died shortly after birth were studied 2 days and 7 weeks before delivery, respectively.

Table IV provides calculations of sensitivity, specificity, and positive and negative predictive values for the

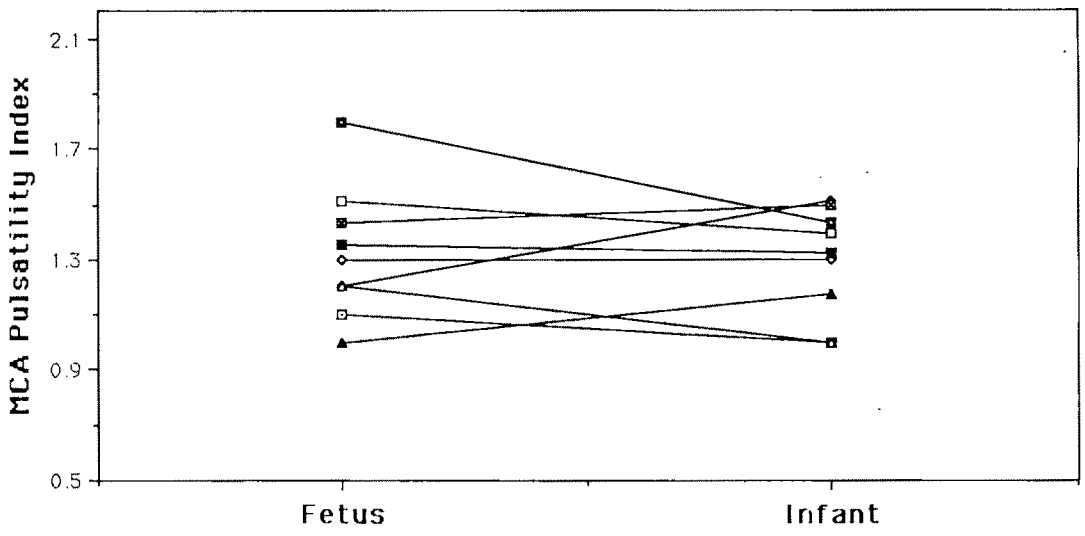


Fig. 3. Middle cerebral artery *(MCA)* pulsatility index values before delivery and at 1 month after delivery in nine normal infants ($p > 0.05$).

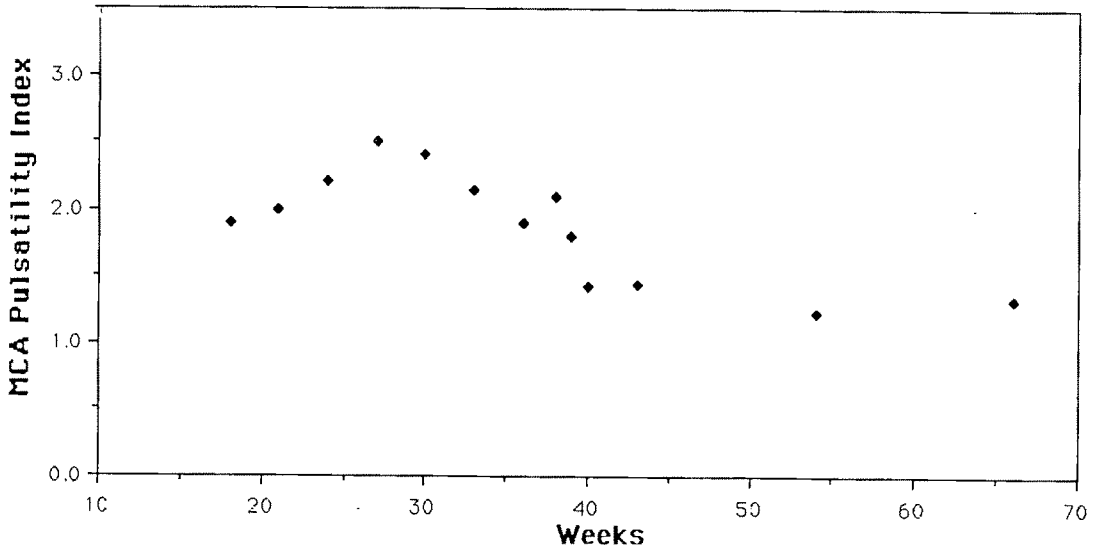


Fig. 4. Middle cerebral artery *(MCA)* pulsatility index values in one infant studied from 16 weeks' gestation to 24 weeks after delivery.

middle cerebral artery pulsatility index below the normal range and adverse perinatal outcome.

Table V provides data on the six perinatal deaths.

The rate of cesarean sections was 42% in the SGA fetuses with normal middle cerebral artery pulsatility index and 55% in the SGA fetuses with abnormal middle cerebral artery pulsatility index. Six of the pregnant women of group 2 had hypertension during their pregnancy.

Comment

Wladimiroff et al.[1] reported that the pulsatility index of the fetal internal carotid artery did not change significantly between 26 weeks' gestation and the term of pregnancy. They suggested that IUGR fetuses could undergo a brain-sparing effect, similar to that reported in the animal literature. Successively, Van Den Wijngaard et al.[5] showed that the pulsatility index of the fetal internal carotid artery decreases during gestation. These authors attributed this change to the relatively low oxygen observed by Soothill et al.[4] in the umbilical artery at the end of pregnancy.

Vyas et al.[14] studied the middle cerebral artery in normal and hypoxemic fetuses and reported that the end-diastolic velocities were absent in 11 of 42 normal fetuses at 18 to 25 weeks' gestation and in 12 of 50

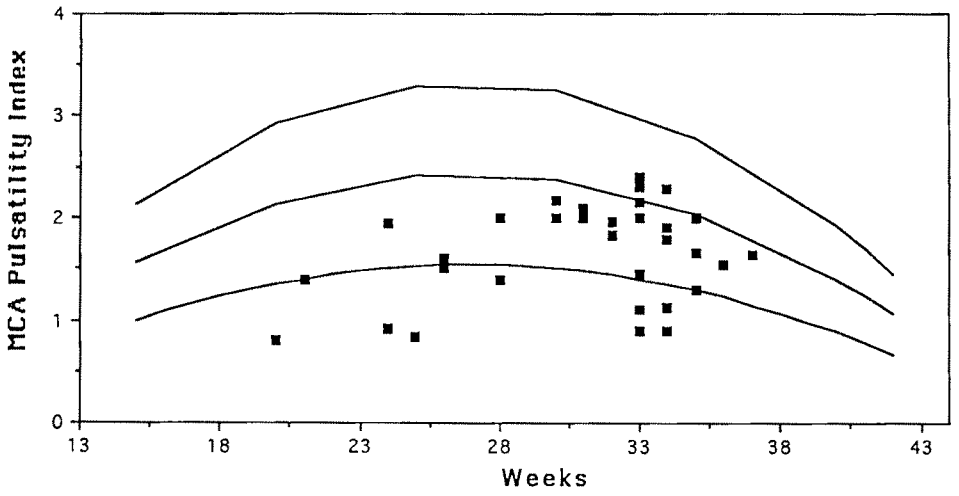


Fig. 5. Middle cerebral artery *(MCA)* pulsatility index of 33 SGA fetuses *(squares)* plotted on reference range. The *large rectangle* at 31 weeks' gestation represents 3 pulsatility index values.

Table III. Middle cerebral artery pulsatility index standard curve (cross-sectional)

Gestational age (wk)	*Lower limit**	*Predicted value*	*Upper limit†*
15	0.99	1.57	2.14
16	1.08	1.71	2.33
17	1.16	1.83	2.51
18	1.23	1.95	2.67
19	1.30	2.05	2.81
20	1.35	2.14	2.93
21	1.40	2.22	3.04
22	1.44	2.29	3.13
23	1.48	2.34	3.20
24	1.51	2.38	3.26
25	1.52	2.41	3.30
26	1.54	2.43	3.32
27	1.54	2.44	3.33
28	1.54	2.43	3.32
29	1.52	2.41	3.30
30	1.50	2.38	3.26
31	1.48	2.34	3.20
32	1.44	2.28	3.12
33	1.40	2.21	3.03
34	1.35	2.13	2.92
35	1.29	2.04	2.79
36	1.22	1.94	2.65
37	1.15	1.82	2.49
38	1.07	1.69	2.32
39	0.98	1.56	2.13
40	0.89	1.40	1.92
41	0.78	1.24	1.70
42	0.67	1.06	1.45

PI, Pulsatility index; *GA,* gestational age. PI $= -1.9763 + (0.32737\ GA) + (-0.00611\ GA^2)$.

*Predicted value − (2 × 0.184 × Predicted value).

†Predicted value + (2 × 0.184 × Predicted value).

fetuses at 26 to 33 weeks. They reported that maximum reduction in pulsatility index is reached when the fetal Po_2 is 2 to 4 SD below normal for gestation. When the oxygen deficit is greater there is a tendency for the pulsatility index to rise; this presumably reflects the development of brain edema.

Veille and Cohen[15] performed Doppler studies of the middle cerebral artery on 17 fetuses who were SGA at the time of delivery. Results were compared with a group of 25 fetuses who had a normal weight for gestational age at the time of delivery. No difference was found in either the systolic/diastolic ratio[16] or the Pourcelot index[17] of the middle cerebral artery.

It is possible that the physiologic fetal hypoxemia reported by Soothill et al.[4] at the end of gestation could contribute to the low pulsatility index observed in this period of gestation. However, we have observed that the low pulsatility index found in nine normal fetuses late during pregnancy does not change significantly after delivery. Additionally, our study indicates that the pulsatility index of the middle cerebral artery is lowest between 15 and 20 weeks' gestation and at the end of gestation. To postulate another explanation for the low pulsatility index observed in the normal fetus at the end of gestation, the factors that could influence the pulsatility index in the cerebral circulation must be examined. The pulsatility index of the middle cerebral artery is decreased in conditions that suggest a decreased cerebral impedance to blood flow caused by either mechanical factors[18] or fetal hypoxemia such as that observed in IUGR fetuses[1] and in the anemic fetus after intravascular transfusion.[3] Therefore our results could indicate a decreased cerebral vascular impedance to blood flow during these two periods of gestation.

That reasonable agreement was obtained between the results of the longitudinal and cross-sectional studies in normal fetuses supports the concept that this pattern of change in the pulsatility index of the middle cerebral artery is physiologic. An explanation for lower vascular impedance during early and late pregnancy

Table IV. Predictive validity of middle cerebral artery pulsatility index below the normal range for gestational age for adverse perinatal outcome

	Disease state				
	*Adverse perinatal outcome**		*No adverse perinatal outcome*		
Test result	*No.*	*%*	*No.*	*%*	*Total*
Positive (Abnormal)	6	66.6	3	33.3	9
Negative (Normal)	4	16.6	20	83.3	24
TOTAL	10	30.3	23	69.7	33

Sensitivity, 60%; specificity, 87%; positive predictive value, 67%; negative predictive value, 83%.

*Admission to neonatal intensive care unit for >12 hours or perinatal death.

Table V. Perinatal deaths

Patient	*Middle cerebral artery pulsatility index*	*Gestational age (wk)*	*Diagnosis*	*Time of death*
1	0.80	20	IUGR	Stillbirth
2	1.40	21	IUGR	Neonatal
3	0.92	24	IUGR-PIH	Neonatal
4	0.84	25.2	IUGR-LA	Stillbirth
5	1.70	26	IUGR	Stillbirth
6	1.96	36	IUGR-Hyd	Neonatal

PIH, Pregnancy-induced hypertension; *LA,* lupus anticoagulant; *Hyd,* hydramnios.

may be found in studies of the development of the human brain. Dobbing and Sands[19] studied human fetal brains; they estimated total cell numbers by chemical analysis of deoxyribonucleic acid–phosphorus in the forebrain, cerebellum, and brain stem. These authors calculated the whole brain values as the sum. They assumed that total tissue deoxyribonucleic acid represented total number of cells. They demonstrated that in the human species there are two periods of major cellular multiplication: one at 15 to 20 weeks' gestation and one that commences in the third trimester and probably ends in the second year of postnatal life. We have observed a lower pulsatility index in the middle cerebral artery during these two periods of increased deoxyribonucleic acid synthesis. It can be speculated that during these two periods of major cellular multiplication there is an increased metabolic requirement in the human fetal brain and a lower cerebral vascular impedance to blood flow.

Our results are in conflict with those reported by Vyas et al.[14] because we have observed the end-diastolic velocity in the middle cerebral artery velocity waveforms in almost all of the fetuses. This discrepancy may be caused by the use of different high-pass filters: 125 Hz in their study, 0 to 50 Hz in the current study. We think it is important to use a low-frequency high-pass filter when studying the middle cerebral artery because of the possibility of missing the diastolic component of the waveforms, which will affect the pulsatility index.

To date, studies of the fetal cerebral circulation have been directed mainly at recognizing IUGR fetuses. An IUGR fetus can be either a fetus with low growth rate caused by chronic hypoxia at risk for adverse perinatal outcome or a small, normally growing baby. Animal and human experiments have shown that the brain-sparing effect is present in conditions of fetal hypoxemia.[14, 20] It is reasonable to assume that the brain-sparing effect is present in the fetus with low growth rate and not in the normal small baby. The results of the SGA fetuses reported in this study indicate that in some SGA fetuses there is a pulsatility index value below the normal range, which suggests a brain-sparing effect. The difference between the fetuses with normal versus abnormal middle cerebral arterial pulsatility index may be clinically important. The fetuses with abnormal pulsatility index had a greater incidence of adverse perinatal outcome.

Recently we proposed that the brain-sparing effect is transitory,[21] as reported during prolonged hypoxemia in animal experiments,[22] and the overstressed human fetus loses the brain-sparing effect. This hypothesis is further supported by the recent findings of Vyas et al.[14] In our series three SGA fetuses with normal middle cerebral artery pulsatility indexes died, the first in utero and the other two shortly after birth. It is possible that these fetuses had a brain-sparing effect during the pregnancy that was not present when we studied the fetuses.

As our study indicates, the brain-sparing effect can be present early in pregnancy (i.e., 20 weeks). This can be clinically important because of the possibility of early recognition of the fetus at higher risk for adverse perinatal outcome.

In conclusion, our study shows that (1) the pulsatility index of the middle cerebral artery of the normal human fetus has a parabolic pattern during pregnancy; (2) a physiologic lower pulsatility index value in the middle cerebral artery of the normal human fetus at 15 to 20 weeks' gestation and late in the third trimester is associated with an increased deoxyribonucleic acid synthesis in the fetal brain; (3) the pulsatility index of the middle cerebral artery after birth in nine normal fetuses did not change significantly from the pulsatility index obtained at the end of gestation; (4) a brain-sparing effect can be present early during gestation (i.e., 20 weeks); and (5) an abnormal pulsatility index of the middle cerebral artery in the SGA fetus is associated with worse fetal outcome.

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Inferior vena cava flow velocity waveforms in appropriate- and small-for-gestational-age fetuses

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Rome and Ancona, Italy

Reference ranges of inferior vena cava flow velocities were constructed from a cross-sectional study of 118 appropriate-for-gestational-age fetuses of 18 to 40 weeks of gestation. Blood flow velocity waveforms were recorded with color and pulsed Doppler equipment. Peak velocities and time velocity integrals were measured from inferior vena cava during systole, early diastole, and atrial contraction. The systolic-to-diastolic ratios between the peak velocities and time velocity integrals were calculated, and the reverse flow with atrial contraction was quantified as the percentage of forward flow (percentage of reverse flow). Recordings were also obtained from 79 small-for-gestational-age fetuses free of structural and chromosomal abnormalities, divided into three groups according to umbilical artery velocity waveforms: normal pulsatility index values (group 1, $n = 26$), pulsatility index >95th percentile of our reference limits but presence of end-diastolic velocities (group 2, $n = 33$), and absence of end-diastolic velocities (group 3, $n = 20$). Fourteen fetuses of groups 2 and 3 were also studied at weekly intervals until the onset of antepartum late heart rate decelerations. In appropriate-for-gestational-age fetuses no changes were evident in peak velocities and time velocity integrals ratios, whereas the percentage of reverse flow significantly decreased with gestation. No significant differences were found between these values and those obtained in small-for-gestational-age fetuses of group 1. A significant increase of peak velocities and time velocity integrals ratios and of percentage of reverse flow was evidenced in fetuses of both group 2 and 3. The fetuses of groups 2 and 3 with a percentage of reverse flow above the 95% confidence interval showed a poorer perinatal outcome when compared with the fetuses of the same groups but with values inside the normal range. In the 14 fetuses longitudinally followed up until the onset of late heart rate decelerations a significant and progressive increase of peak velocities and time velocity integrals ratios and percentage of reverse flow was evidenced in spite of minimal changes in the pulsatility index from both umbilical artery and different peripheral fetal vessels. This study presents evidence that in small-for-gestational-age fetuses with abnormal Doppler-measured placental resistance the modified flow velocity patterns in the inferior vena cava seem to deteriorate progressively with advancing gestation. (Am J Obstet Gynecol 1992;166:1271-80.)

Key words: Venous blood flow, Doppler ultrasonography, fetal growth retardation

Abnormal inferior vena cava flow velocity waveforms have been described in different fetal pathologic conditions, including anemia,[1] nonimmune hydrops,[2] and arrythmias.[3, 4] Furthermore, abnormalities of inferior vena cava waveforms, were also found in growth-retarded fetuses, characterized by extreme compromise as expressed by the absence of end diastolic flow in umbilical artery.[3]

There is no account, however, on the longitudinal changes occurring in inferior vena cava blood flow patterns with the progression of normal pregnancy and on their modifications in growth-retarded fetuses with different degrees of compromise.

This study uses Doppler ultrasonography to establish reference ranges of velocity waveforms in the inferior vena cava of healthy fetuses from 18 to 40 weeks of gestation. Furthermore, it investigates possible modifications in the flow velocity waveforms of small-for-gestational-age (SGA) fetuses and relates the Doppler findings to the severity of fetal compromise.

Material and methods

Subjects. Reference ranges of inferior vena cava velocity waveforms were constructed from the cross-sectional study of 118 appropriate-for-gestational-age (AGA) fetuses (i.e., birth weight ranging between the 10th and 90th percentile). These fetuses were recruited from the routine antenatal clinic of our institution. All pregnancies were singleton and dated by certain last menstrual period and early second-trimester ultraso-

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6/1/34060

Table I. Characteristics of fetuses investigated (mean ± 1 SD or number and percent)

		SGA			Significance*	
	AGA (n = 118)	Group 1 (n = 26)	Group 2 (n = 33)	Group 3 (n = 20)	AGA vs group 1	AGA vs group 2
Gestational age at Doppler recordings (wk)	29.6 ± 5.1	30.3 ± 2.6	31.0 ± 2.5	28.3 ± 5.2	NS	NS
Umbilical artery pulsatility index (Δ value)	−0.12 ± 0.99	0.33 ± 0.94	3.04 ± 1.13	4.12 ± 1.62	NS	$p \leq 0.001$
Descending aorta pulsatility index	1.94 ± 0.21	1.97 ± 0.26	2.46 ± 0.41	2.64 ± 0.55	NS	$p \leq 0.001$
Renal artery pulsatility index (Δ-value)	0.03 ± 0.83	0.21 ± 0.96	2.24 ± 1.45	3.19 ± 1.84	NS	$p \leq 0.001$
Internal carotid artery (Δ-value)	−0.06 ± 0.87	0.27 ± 0.75	−2.67 ± 1.44	−3.19 ± 1.46	NS	$p \leq 0.001$
Middle cerebral artery pulsatility (Δ-value)	0.12 ± 0.88	0.31 ± 0.92	−2.95 ± 1.21	−3.32 ± 1.33	NS	$p \leq 0.001$
Gestational age at birth (wk)	39.7 ± 1.9	38.4 ± 2.5	34.8 ± 3.2	32.1 ± 4.2	NS	$p \leq 0.001$
Birth weight (gm)	3654 ± 412	2320 ± 316	1635 ± 521	1135 ± 363	$p \leq 0.001$	$p \leq 0.001$
Umbilical artery pH	—	7.251 ± 0.029	7.223 ± 0.034	7.188 ± 0.042	—	—
Antepartum late heart rate decelerations (%)	0 (0)	0 (0)	13 (39.4)	14 (70.0)	NS	$p \leq 0.001$
Cesarean section (%)	9 (7.6)	3 (11.5)	28 (84.5)	20 (100)	NS	$p \leq 0.001$
5 min Apgar score <7	0 (0)	0 (0)	9 (27.2)	9 (45.0)	NS	$p \leq 0.001$
Neonatal mortality	0 (0)	0 (0)	1 (3.0)	4 (20.0)	NS	NS

Because pulsatility index values obtained from umbilical artery, renal artery, internal carotid artery, and middle cerebral artery change with gestation, data are expressed as number of standard deviations by which they differ from their normal mean for gestation (Δ-values). *NS*, Not significant.

*Statistical significance was evaluated by unpaired t test and Fisher's exact test.

nography. Ultrasonographic examination at the time of Doppler studies showed all the fetuses to be structurally normal and of appropriate size for gestational age. Infants were born in good condition; their characteristics are reported in Table I.

Inferior vena cava velocity waveforms were also studied in 79 SGA fetuses. Entry criteria required (1) singleton gestation accurately dated by early second-trimester fetal biparietal diameter mesurement and (2) abdominal circumference <5th percentile of our reference limits (fetuses with a gestational age <28 weeks) or ultrasonographically estimated fetal weight (fetuses with a gestational age ≥28 weeks) <5th percentile for our population. The diagnosis of SGA (i.e., birth weight <5th percentile and newborn free of structural abnormalities) was confirmed postnatally in all included subjects. SGA fetuses were divided into three groups according to the results obtained at the Doppler examination of the umbilical artery as follows: (1) group 1, normal pulsatility index in the umbilical artery with respect to our reference limits for gestation[5] (n = 26); (2) group 2, abnormal pulsatility index in the umbilical artery (i.e., >95th percentile) but velocity waveforms characterized by the presence of end-diastolic velocities (n = 33); (3) group 3, abnormal pulsatility index in the umbilical artery and absent end-diastolic velocities (n = 20) (none of these fetuses had reversed flow in the umbilical artery).

The characteristics of these three groups of fetuses are reported in Table I. Although umbilical artery pulsatility index values were provided to the clinicians managing the cases, decisions on delivery were not undertaken on the basis of Doppler data.

Some of the SGA fetuses with an abnormal pulsatility index in the umbilical artery were also studied longitudinally at weekly intervals until delivery. To obtain a homogeneous group, we retained the data of the 14 fetuses (10 of group 2 and four of group 3) in whom antepartum late heart rate decelerations developed and who had been studied on at least four occasions 1 week apart (longitudinal study). In these fetuses heart rate monitoring was performed daily for a minimum of 45 minutes from the time of diagnosis onward. At the onset of late decelerations during Braxton Hicks contractions, an elective cesarean section was performed.

All the patients gave their informed consent to participate in the study.

Doppler recordings. Commercially available Ansaldo Hitachi Esacord 81 color Doppler equipment (Ansaldo Hitachi, Genoa, Italy) with 3.5 or 5 MHz convex probes was used for all measurements. In addition to the color flow mapping function, the machine was equipped with pulsed Doppler that had a carrier frequency ranging from 2.5 to 5 MHz. After identification of the inferior vena cava in a longitudinal plane on a sagittal view of the fetal trunk, the color flow mapping

Significance*			
AGA vs group 3	*Group 1 vs group 2*	*Group 1 vs Group 3*	*Group 2 vs group 3*
NS	NS	NS	NS
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	NS
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.05$
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	NS
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	NS
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.05$
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$
—	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.005$
$p \leq 0.001$	$p \leq 0.002$	$p \leq 0.001$	$p \leq 0.01$
$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	NS
$p \leq 0.001$	$p \leq 0.005$	$p \leq 0.002$	NS
$p \leq 0.005$	NS	$p \leq 0.05$	NS

function was superimposed. On the basis of previously reported techniques in the first 70 cases (45 AGA and 25 SGA) the pulsed Doppler sample volume was placed both at the inlet of inferior vena cava in the right atrium[3] and in the portion of inferior vena cava between the entrance of the renal vein and the ductus venosus.[2,4] A good coefficient of correlation was found between the measurements performed at the two sampling sites ($r > 0.84$). The latter sampling site was selected for the further examinations because of the significantly lower angle (mean ± 1 SD 21 ± 7 vs 39 ± 6 degrees, $p \leq 0.001$) between the Doppler beam and the direction of blood flow, as estimated on the color image.

Flow velocity waveforms were recorded from each fetus during periods of fetal rest without breathing movements. All the recordings were performed by one of the authors (G.R.).

Permanent records of all the velocity waveforms were obtained from the videotape by means of a strip chart recorder. The printouts were labeled with random numbers. The observer involved in the later analysis was not informed of the sequence of the printouts. Three components were identified in velocity waveforms as described by Reed et al.[5]: (1) systole (S wave), (2) early diastole (D wave), and (3) reverse flow during atrial contraction (A wave) (Figs. 1 and 2). Estimates of the peak velocities and time velocity integrals of the three components were made on 10 uniform and consecutive heart cycles with the digitizing tablet of a computer (Cardio 800, Kontron, Oxford). The following indices were calculated from these measurements: (1) ratio between the peak velocity of S and D waves, (2) ratio between time velocity intervals of S and D waves, and (3) percentage of reverse flow quantified as the percent of time velocity intervals during A wave with respect to total forward time velocity intervals.

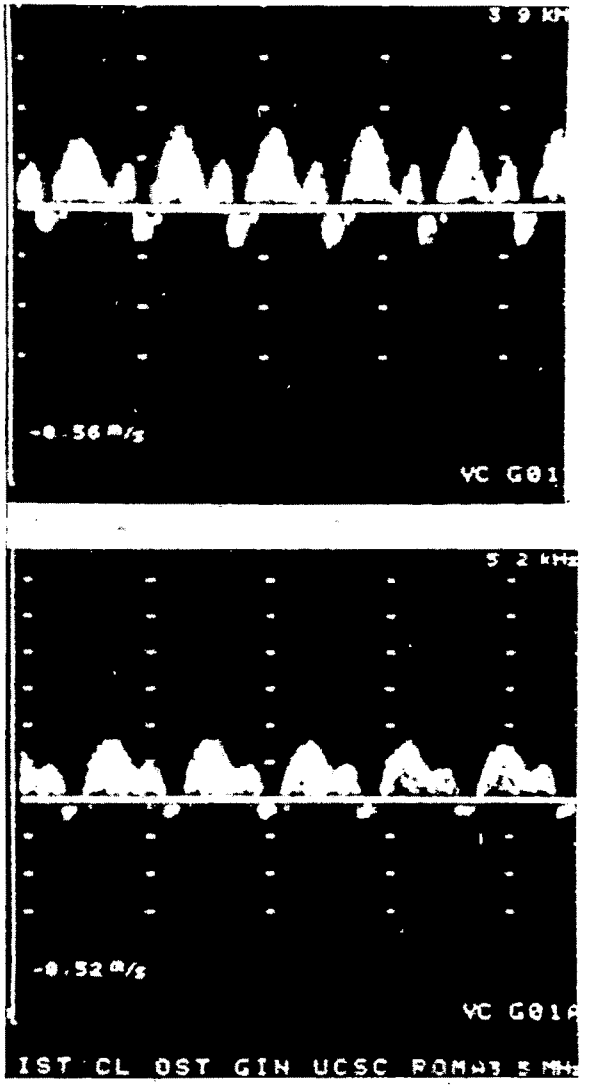


Fig. 1. Flow velocity waveforms from fetal inferior vena cava in AGA fetuses at 20 *(top)* and 39 *(bottom)* weeks of gestation with percentage of reverse flow *(bottom channel)* of 18.5 in former and 4.3 in latter.

Intraobserver variability was determined by examining 19 fetuses for two consecutive days. The maximum difference in the indices considered in the same fetus was 8.9% (mean 5.4%).

In all the fetuses studied blood flow velocity waveforms were also recorded at the level of the umbilical artery, middle cerebral artery, descending aorta, and renal artery with previously described techniques,[5] the pulsatility index was calculated as a measure of vascular impedance.[6]

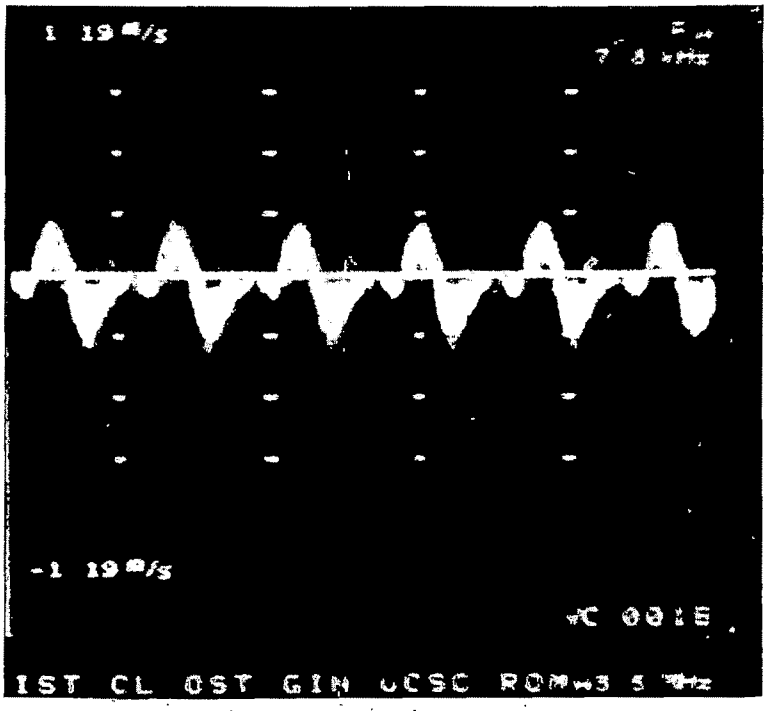


Fig. 2. Flow velocity waveforms from fetal inferior vena cava in SGA fetus of group 3 at 29 weeks of gestation with percentage of reverse flow *(top channel)* of 38.3.

Data analysis. Regression analysis was used to establish the mean and individual 95% confidence intervals of the relationship of the indices considered and gestation. Data are presented as mean ± 1 SD. For measurements changing with gestation data are also expressed as number of standard deviations by which they differ from their normal mean for gestation (Δ-values). Differences were evaluated by means of one sample and unpaired t test, Wilcoxon rank-sum test, and Fisher's exact test. The longitudinal changes in Doppler indexes were evaluated by the analysis of variance for repeated measurements. Regression analysis was used to test the relationships between the variables considered. Differences yielding $p \leq 0.05$ were considered significant.

Results

AGA fetuses. These fetuses showed a slight but not significant linear increase with gestation of the peak velocity S/D ratio (Fig. 3, $r = 0.191$, $p = 0.07$, mean value 1.757, SD 0.334). Similarly, no significant changes with gestation were found for the S/D time velocity intervals ratio (Fig. 3, $r = 0.091$, mean value 2.612, SD 0.997), whereas the percentage of reverse flow significantly and linearly decreased with gestation (Fig. 3, constant 24.671, slope −0.499, SD 2.557, $r = 0.775$, $p \leq 0.001$).

SGA fetuses, group 1. No significant differences were found in peak velocity S/D ratio (mean value = 1.63, SD 0.32, $t = 1.737$, not significant), S/D time velocity intervals ratio (mean value 2.62, SD 0.88, $t = 0.146$, not significant) and percentage of reverse flow (mean value 8.26, SD 2.41, Δ-value 0.09 SD, $t = 0.474$, not significant) when the values obtained in SGA fetuses with normal umbilical artery pulsatility index values were compared with those of AGA fetuses (Fig. 4).

SGA fetuses, group 2. A significant increase in peak velocity S/D ratio (mean value 1.945, SD 0.45, $t = 2.514$, $p \leq 0.01$), S/D time velocity intervals ratio (mean value 3.05, SD 1.51, $t = 1.934$, $p \leq 0.05$), and percentage of reverse flow (mean value 11.88, SD 6.04, Δ-value 1.36 SD; $t = 3.125$, $p \leq 0.005$) was found in this group of SGA fetuses when compared with AGA fetuses (Fig. 5). No relationships were evident between these indices and the pulsatility index values (absolute values and Δ-values) from umbilical artery, descending aorta, renal artery, and middle cerebral artery. Moreover, when the nine SGA fetuses with percentage of reverse flow values >95% confidence interval were compared with the remaining 24 SGA fetuses, a higher incidence of antepartum late heart rate decelerations (7/9 vs 6/24, Fisher's exact test $p \leq 0.01$), a shorter time interval between the recordings and delivery (median 11 days, range 3 to 21 vs 32, range 8 to 54, Wilcoxon test $p \leq 0.01$), and a lower umbilical artery pH (7.181 ± 0.022 vs 7.228 ± 0.021, $p \leq 0.001$) were evident. In addition, no differences were found between these two subgroups of fetuses either in birth weight (1442 ± 576 vs 1716 ± 354 gm, not significant) or in

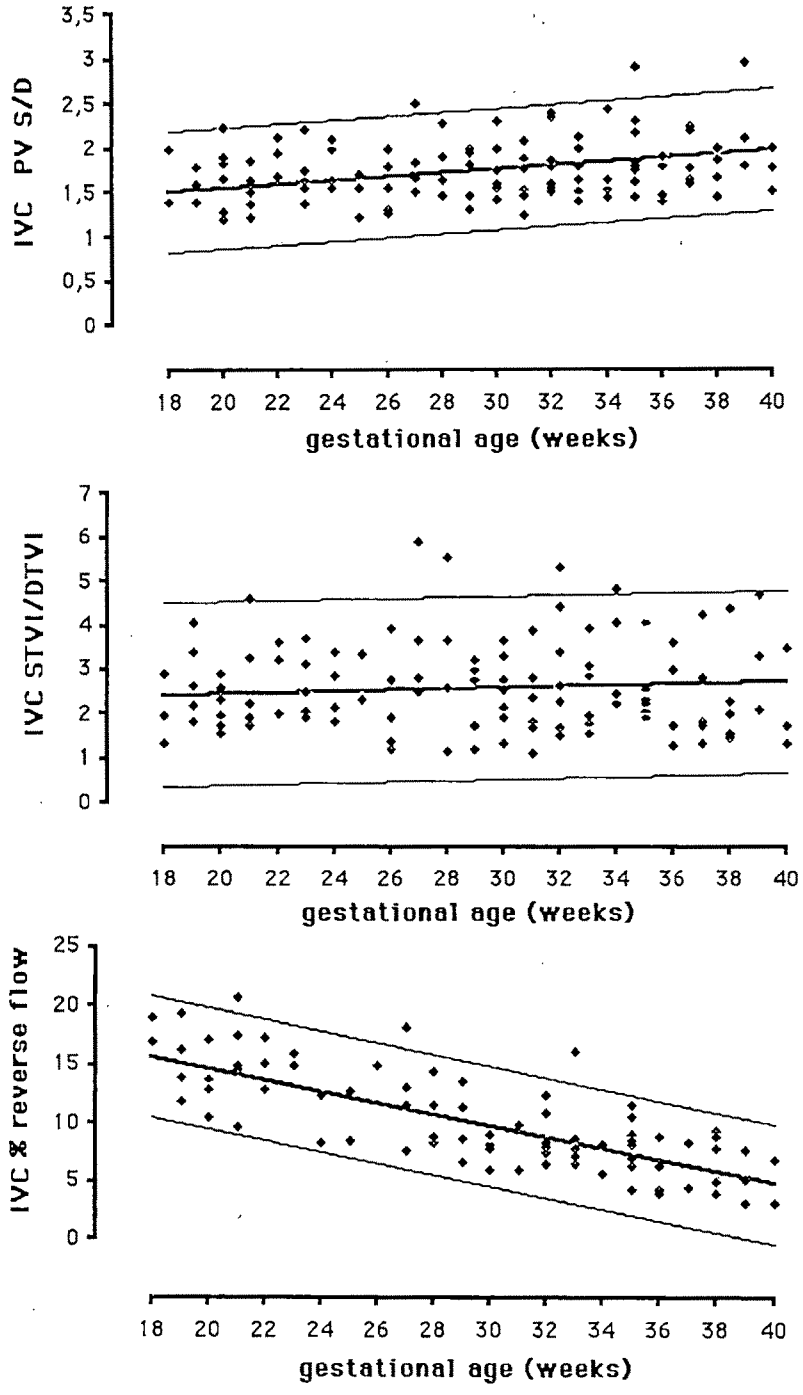


Fig. 3. Reference range (mean and linear regressions of individual 95% confidence intervals) with gestation of inferior vena cava *(IVC)* S/D ratios of peak velocities *(PV S/D)* and time velocity integrals *(STVI/DTVI)* and of percentage of reverse flow during atrial contraction.

pulsatility index values obtained from the different arterial districts considered. Moreover, the single case of neonatal death (intraventricular hemorrhage) that occurred in this group had a percentage of reverse flow value >95% confidence interval.

SGA fetuses, group 3. Highly significant differences were found in the comparison of SGA fetuses with AGA fetuses for peak velocities S/D ratio (mean value 2.39, SD 1.21, $t = 4.721$, $p \leq 0.001$), S/D time velocity intervals ratio (mean value 4.18, SD 2.84; $t = 5.294$, $p \leq 0.001$), and percentage of reverse flow (mean value 21.86, SD 10.53, Δ-value 5.06 SD, $t = 14.754$, $p \leq 0.001$) values (Fig. 5). Similarly, with group 2 fetuses no relationships were evident between inferior vena

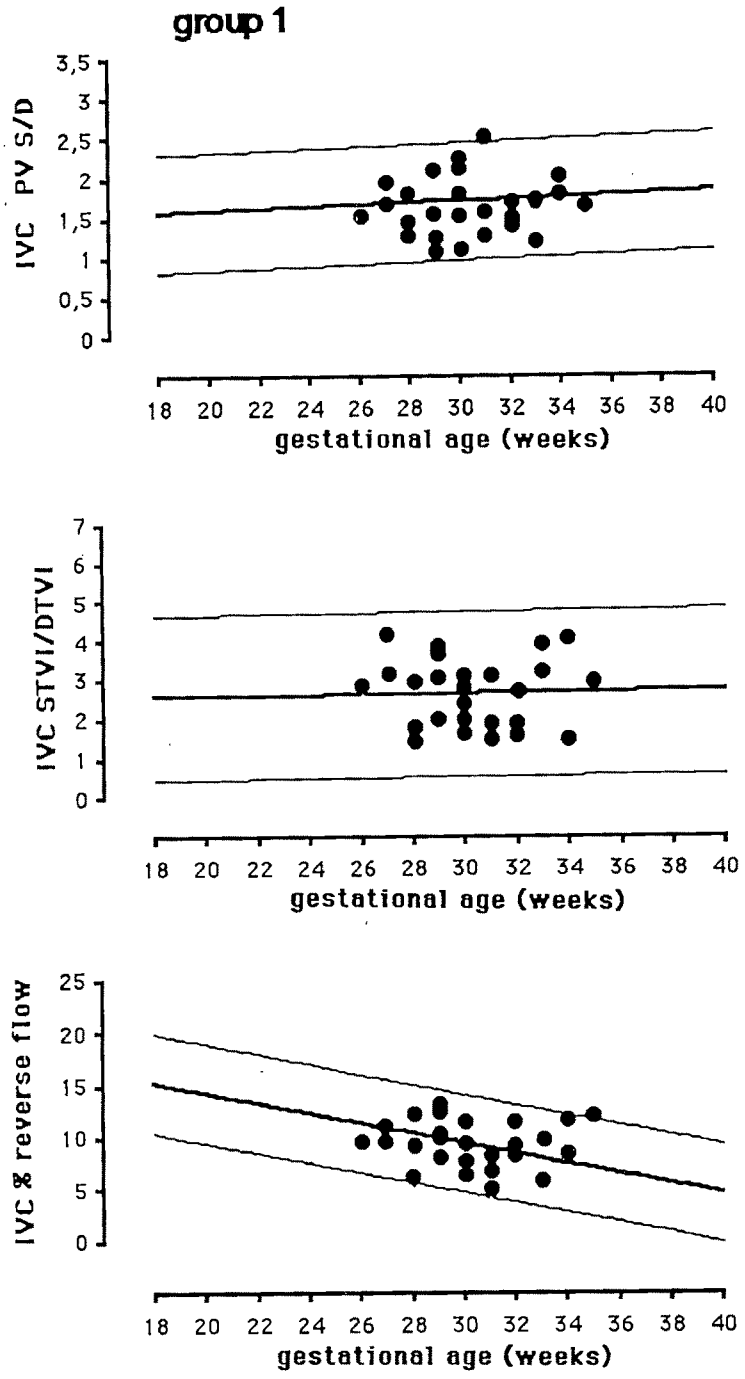


Fig. 4. Inferior vena cava *(IVC)* peak velocities *(PV)* S/D and S/D time velocity intervals ratios *(STVI/DTVI)* and percentage of reverse flow values of 33 SGA fetuses of group 1 (normal umbilical artery pulsatility index) plotted on reference range.

cava indices and pulsatility index values from arterial beds. In 14 fetuses of this group the percentage of reverse flow was >95th confidence interval, whereas the remaining six fetuses showed values inside the reference limits. When these two subgroups were compared, fetuses with abnormal percentage of reverse flow values showed a shorter interval between the recordings and delivery (median 5 days, range 0 to 11 vs 26, range 4 to 36, Wilcoxon test $p \leq 0.01$) and a higher incidence of antepartum late heart rate decelerations (12/14 and 2/6, Fisher's exact test $p \leq 0.03$). Three of the four neonatal deaths occurred in the fetuses with abnormal percentage of reverse flow. No further significant differences were noted between the two subgroups.

Longitudinal study. Each SGA fetus longitudinally

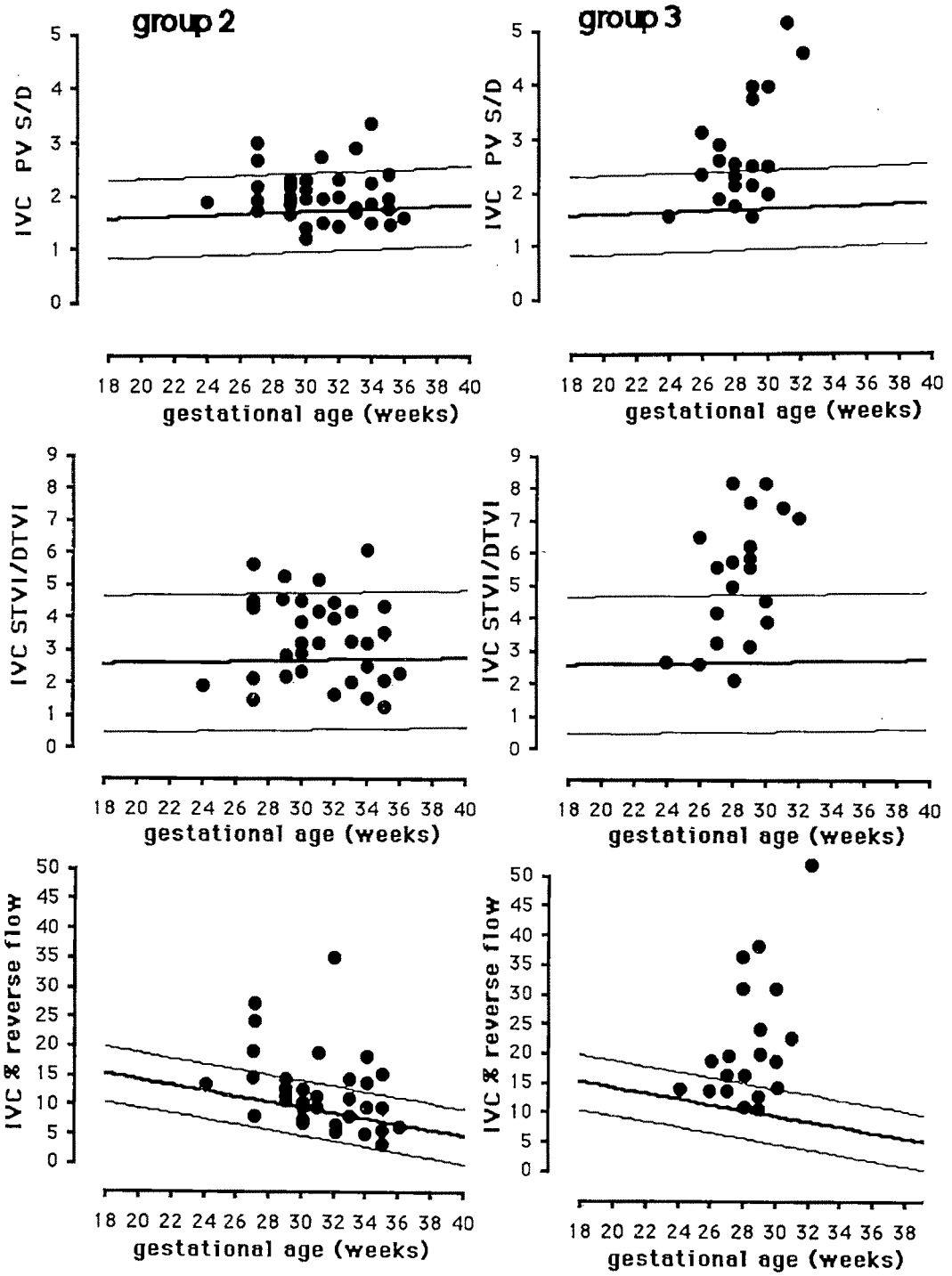


Fig. 5. Inferior vena cava *(IVC)* peak velocities *(PV)* S/D and S/D time velocity intervals ratios *(STVI/DTVI)* and percentage of reverse flow values of SGA fetuses of group 2 (increased umbilical artery pulsatility index and end-diastolic velocities present, *left side*) and group 3 (increased umbilical artery pulsatility index and absent end-diastolic velocities, *right side*) plotted on reference range.

studied underwent a mean of 4.71 Doppler recordings (range 4 to 7). Gestational age at the first study was 28.3 ± 2.5 and 32.1 ± 2.3 weeks at birth. Mean birth weight was 1379 ± 342 gm and mean umbilical artery pH was 7.218 ± 0.041. Although two of the fetuses of group 2 developed absent end-diastolic velocities in the umbilical artery, minimal overall changes were evidenced in pulsatility index from the umbilical artery ($F = 1.912$, $p \leq 0.05$) and middle cerebral artery ($F = 1.893$, $p < 0.01$). No significant changes were

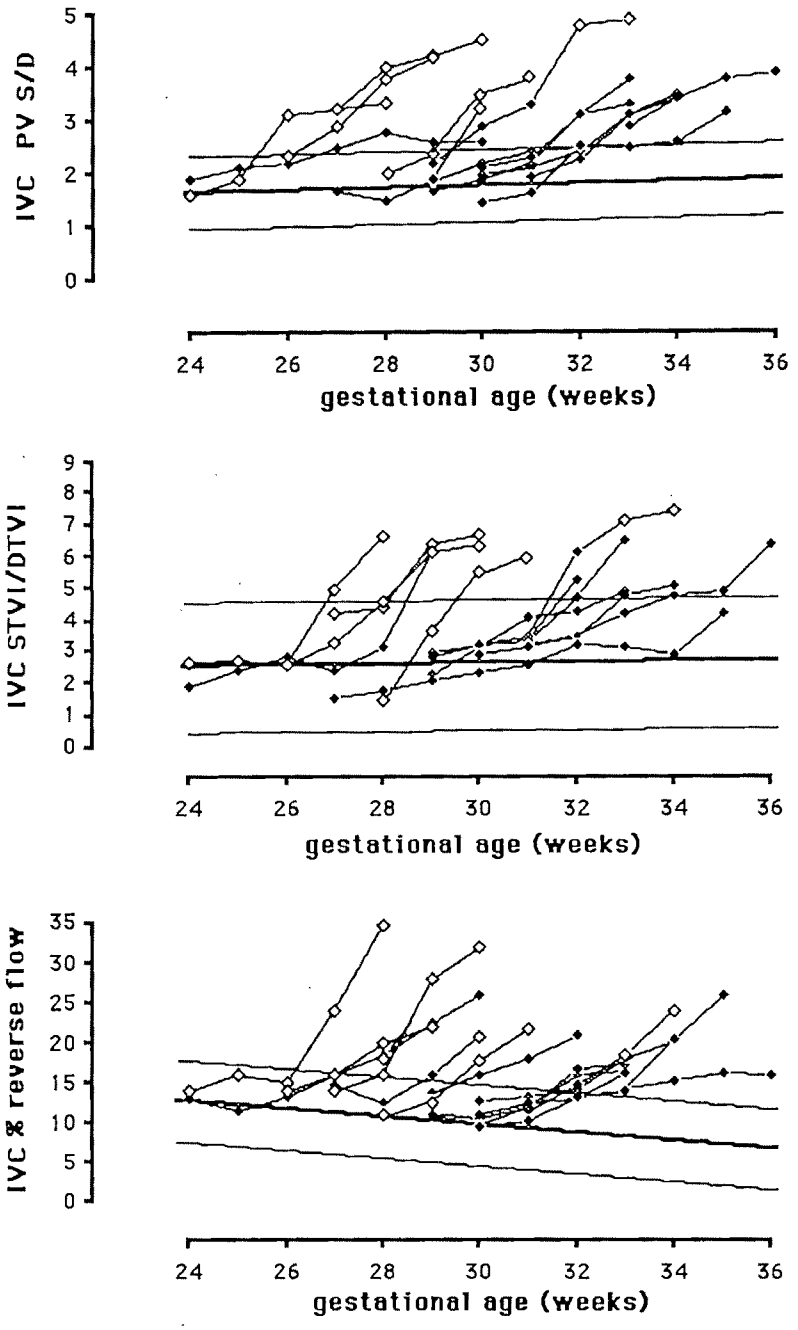


Fig. 6. Individual changes of peak velocities *(PV)* S/D and S/D time velocity intervals ratios *(STVI/DTVI)* and percentage of reverse flow values in 14 SGA fetuses studied longitudinally plotted on reference ranges. Values of each individual patient are connected by lines. *Open diamonds*, Absent end-diastolic velocities in umbilical artery, *IVC*, inferior vena cava.

found in descending aorta ($F = 0.348$, not significant;) and renal artery ($F = 0.879$, not significant) pulsatility index values. When analysis of variance was applied to inferior vena cava indexes a significant increase of peak velocity S/D ratio ($F = 6.123$, $p \leq 0.001$), the S time velocity intervals/D time velocity intervals ratio ($F = 7.423$, $P \leq 0.001$), and the percentage of reverse flow ($F = 14.23$, $p \leq 0.001$) was evident with advancing gestation (Fig. 6). No significant relationships were noted between these changes in inferior vena cava and the concomitant modifications of the pulsatility index from the umbilical artery and the middle cerebral artery.

Comment

Flow velocity patterns in the inferior vena cava are inversely related to the modifications of right atrial pressure.[7] In the human fetus the inferior vena cava blood

flow has a pulsatile pattern similar to that described in animal fetuses or in adults.[7, 8] In these models it has been demonstrated that the first forward wave begins to increase with atrial relaxation, reaches the peak during ventricular systole, and then falls to reach the nadir at the end of ventricular systole. The second forward wave occurs during early diastole, whereas a reverse flow is usually present in late diastole with atrial contraction. Simultaneous recordings of the inferior vena cava and descending aorta velocity waveforms have also allowed confirmation of the timing of these events in the human fetus.[3]

In normal pregnancy the peak velocities and the time velocity intervals values obtained during systole in the inferior vena cava exceeded the early diastolic values resulting in ratios >1. The absence of changes of the systolic-to-diastolic ratios with gestation suggests a constant filling pattern during systole and early diastole. Moreover, a significant decrease of reverse flow during atrial contraction was evident with advancing gestation. In the fetal lamb and in adults (animals and humans) the percentage of reverse flow is related to the pressure gradient present between the right atrium and the right ventricle at end diastole, which is related both to ventricular compliance and to ventricular end-diastolic pressure.[7, 9, 10] The noninvasive nature of the human fetal model does not allow differentiation between these factors, but it can be suggested that both may significantly contribute to the decrease of reverse flow evidenced throughout gestation. Previous studies have demonstrated that the fetal heart is less compliant than the neonatal heart[11] and that a progressive maturation of Doppler-measured indices of ventricular diastolic performance occurs, thus suggesting an improvement of ventricular compliance with advancing gestation.[12, 13] Furthermore, as a consequence of the reduction of placental resistances with gestation[5] it may be suggested that the right ventricle afterload falls. This might result in decreased residual volume and ventricular end-diastolic pressure.

SGA fetuses are a heterogeneous group that includes fetuses that are small because of racial or genetic factors and those that are growth retarded as result of malnutrition caused by uteroplacental insufficiency. The good outcome of the SGA fetuses belonging to group 1 and the normality of the pulsatility index in fetal arterial peripheral vessels make the former etiology for these fetuses likely. The absence of evident modifications in inferior vena cava flow patterns suggests normal cardiac hemodynamics. In particular, the finding of values of percentage of reverse flow similar to those of fetuses matched for gestational age but of larger size is consistent with a normal development of the ventricular filling properties in spite of the reduced growth.

Both the selection criteria and pregnancy outcome strongly suggest that the growth defect of the fetuses of groups 2 and 3 resulted from uteroplacental insufficiency. These fetuses showed an increase of peak velocities and time velocity integral systolic-to-diastolic ratios associated with an increased percentage of reverse flow. These modifications are similar to those induced by hypoxia in instrumented lamb fetuses[7] and confirm recent observations in growth retarded human fetuses characterized by absent end-diastolic velocities in the umbilical artery.[3] These changes are compatible with an impaired cardiac function that may be secondary to different reasons, including a decreased ventricular compliance,[13] a higher end-diastolic ventricular pressure caused by an increased afterload consequent to the increased peripheral and placental resistances,[14, 15] or a reduced right myocardial contractility.[16, 17] As a consequence of these abnormal flow patterns, the return of blood from the placenta to the heart is impaired, thus further reducing the supply of oxygen and nutrients to fetal tissues. This may explain our data on the different outcome of fetuses with similar modifications in arterial vascular districts but different venous flow abnormalities. This hypothesis is supported by the data of Indik et al.[13] who found a different trend in perinatal mortality in fetuses with absent end-diastolic flow in the umbilical artery in the presence of pulsation in the umbilical vein, a situation closely associated with increased value of percentage of reverse flow.

It is noteworthy that in our series an increased reverse flow is present also in approximately 27% of the SGA fetuses of group 2 (presence of end-diastolic velocities in the umbilical artery). In spite of the absence of any differences in pulsatility index values from various different arterial vascular beds or in fetal smallness these fetuses had a higher incidence of antepartum late heart rate decelerations, a shorter time interval between the recordings and delivery, and lower umbilical artery pH values. These findings suggest that among growth-retarded fetuses with uteroplacental insufficiency the study of inferior vena cava velocity waveforms might allow better identification than that with conventional measurements in arterial peripheral vessels, a subgroup of fetuses with a more severe degree of fetal compromise.

The lack of correlation between Doppler-measured arterial resistances and inferior vena cava flow patterns suggests that the degree of cardiac dysfunction necessary to yield abnormal venous patterns in the inferior vena cava is not directly related to arterial vascular resistances but may depend on different factors such as the temporal duration of the hemodynamic alterations. Our longitudinal study allowed us to validate this concept. In spite of the minimal changes in the pulsatility index values in arterial vascular districts there was a progressive deterioration of the inferior vena cava indices considered in the time interval elapsing between

the diagnosis to the onset of abnormal fetal heart rate patterns suggestive of abnormal fetal oxygenation.[19] These findings are compatible with the fall of both cardiac output and aortic and pulmonary peak velocities described in the growth-retarded fetuses longitudinally followed.[20] All these changes may be expressions of the same phenomenon, namely, a progressive deterioration of cardiac function.

Moreover, the advantages of recording inferior vena cava velocity waveforms must be pointed out. Unlike echocardiographic measurements, the indices of inferior vena cava used are angle independent and do not need calculation of vessel area, thus allowing more reproducible and easier recordings.

In conclusion, abnormal flow patterns in the inferior vena cava are present in SGA fetuses as a result of uteroplacental insufficiency. These patterns are unrelated to the abnormalities present in Doppler-measured vascular resistances in fetal arterial vascular beds. However, the noninvasive nature of the human fetal model does not allow for full clarification of the underlying pathophysiologic condition in these abnormal venous velocity patterns. The presence of increased reverse flow in the inferior vena cava in spite of the presence of end-diastolic flow in the umbilical artey may be a warning sign. In fetuses followed up longitudinally until the onset of spontaneous late heart rate decelerations a progressive deviation from the norm of inferior vena cava flow patterns was evidenced, thus suggesting their potential role in the longitudinal monitoring of growth-retarded fetuses. However, an evaluation by means of decision-making techniques (e.g., analysis of receiver-operator characteristic curves) on a larger number of fetuses is required before inferior vena cava flow is used as a clinical test.

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Diathermy loop excision in the management of cervical intraepithelial neoplasia: Diagnosis and treatment in one procedure

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OBJECTIVE: Diathermy loop excision was performed as a new diagnostic-treatment procedure in patients with cervical cytologic diagnoses consistent with an epithelial abnormality.

STUDY DESIGN: A total of 424 patients with cervical cytologic diagnoses consistent with an epithelial abnormality, but macroscopically or colposcopically not consistent with invasive carcinoma, were subjected to diathermy loop excision to diagnose and treat cervical lesions in one procedure.

RESULTS: The diagnostic accuracy rate was 99%. In patients with histologically confirmed grade 3 cervical intraepithelial neoplasia, pretreatment cytologic diagnosis and subsequent histopathologic diagnosis corresponded in 73% of cases. In 91% of all patients the diathermy loop excision was sufficient for complete treatment. Cervical morphologic findings after treatment allowed adequate cytologic follow-up. There was no evidence that diathermy loop excision influenced fertility or pregnancy outcome.

CONCLUSION: Diathermy loop excision is a reliable, well-tolerated, inexpensive, and efficient technique for the management of cervical intraepithelial neoplasia. This outpatient procedure is especially recommended in younger patients, because diathermy loop excision preserves the function of the cervix. (AM J OBSTET GYNECOL 1992;166:1281-7.)

Key words: Diathermy loop, cervical intraepithelial neoplasia, follow-up

Screening for cervical cancer is carried out worldwide by means of cervical smears. Problems associated with cytologic screening of the uterine cervix have been the subject of many studies. In his review article Koss[1] stated that, in spite of the known failures in the screening system, the cervical smear is still an effective tool for cancer detection. The natural history of cervical intraepithelial neoplasia remains a controversial subject. It is generally accepted that grades 1 and 2 cervical intraepithelial neoplasia will either regress, persist or progress to grade 3 cervical intraepithelial neoplasia. Even after several years of persistence, a substantial percentage of grade 3 cervical intraepithelial neoplasia will ultimately progress to invasive carcinoma.[2] Consequently, all patients with grade 3 cervical intraepithelial neoplasia lesions require treatment, and in addition many surgeons in this field also treat grades 1 and 2 cervical intraepithelial neoplasia lesions. In the case of an abnormal cervical smear further investigation is necessary. In general, the management of these patients consists of destruction or complete excision of the entire transformation zone after invasive carcinoma has been excluded by means of histopathologic examination of one or more biopsy specimens.

The colposcopically directed punch biopsy has been accepted as a valuable technique for achieving an accurate histologic diagnosis. Cartier[3] introduced the use of the diathermy loop excision for cervical biopsy and suggested its use as a local (excision) treatment procedure. Prendiville et al.[4] showed diathermy loop excision to be a better technique for performing cervical biopsies when compared with the punch biopsy technique. In 1982 De Haan and Kenemans[5] were among the first to use the diathermy loop as a therapeutic procedure in which the complete transformation zone in patients with cervical intraepithelial neoplasia was removed after a histologic diagnosis was established on the basis of biopsy material.

Histologic examination of the whole transformation zone, obtained by diathermy loop excision, enables the pathologist to make a more accurate diagnosis than is usually possible when only biopsy material is examined. We therefore abandoned the preceding biopsy procedure in our clinic, making use of the diathermy loop excision as a combined diagnostic and therapeutic approach for the management of cervical intraepithelial neoplasia.

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Table I. Age distribution of patients treated by diathermy loop excision (5-year age group, $n = 424$)

	Age (yr)								
	20-24	*25-29*	*30-34*	*35-39*	*40-44*	*45-49*	*50-55*	*55-60*	*60-72*
No.	20	95	112	101	56	19	13	5	3
%	4.7	22.4	26.4	23.8	13.2	4.5	3.1	1.2	0.7

This study analyzed diagnostic accuracy, morbidity, and treatment results of diathermy loop excision during long-term follow-up in a large number of patients with cytologic diagnosis suspicious for cervical intraepithelial neoplasia lesions.

Material and methods

Patients. From January 1982 to January 1988, all patients with cytologic diagnoses consistent with cervical intraepithelial neoplasia lesions or (micro)invasive carcinoma were managed by diathermy loop excision of the entire transformation zone without a prior diagnostic biopsy. All patients underwent colposcopic examination before treatment. Diathermy loop excision was then performed even if the transformation zone was not fully visible. The only exclusion criterion for diathermy loop excision was suspicion of invasive carcinoma on colposcopic examination. A total of 424 patients were entered in this study, the majority being ≤35 years old (Table I).

The cytologic findings before treatment are presented in Table II. In 20 patients smears were suspect for slight dysplasia, moderate dysplasia was suspected in 72 patients and severe dysplasia in 168 patients. Furthermore, smears were consistent with carcinoma in situ in 153 patients, and 11 patients had smears suspicious of (micro)invasive carcinoma. Pretreatment cytologic findings were compared with final histopathologic diagnoses (Table II).

Technique. After colposcopy, paracervical tissue was infiltrated with 10 ml of 1% prilocaine hydrochloride in each side. A rectangular stainless steel diathermy loop excision (0.2 mm, Fig. 1) was chosen according to the extent of the lesion and connected to a diathermy machine (Valleylab SSE 2). After application of iodine solution the entire iodine-negative area was removed. There was no standard procedure for the excision, but usually the posterior lip was removed before the anterior lip and eventually, depending on the extent of the lesion at colposcopic evaluation, a central and sometimes a deeper central excision was performed. If necessary, hemostasis was obtained by coagulation with the loop or a ball electrode. All tissue obtained at diathermy loop excision was submitted for histopathologic examination.

Follow-up. Completeness of treatment was assessed by follow-up cervical smears. Follow-up ranged from 3 to 8 years with a median follow-up of 4.8 years. Within 2 years after treatment, three or more adequate smears were obtained from 375 patients. After three normal smears, a control cytologic examination was usually performed every year. When an abnormal smear was found during follow-up, the diagnostic and treatment procedure was started again. Immediate and long-term morbidity and the influence of diathermy loop excision on fertility and pregnancy outcome were recorded.

Results

Tissue obtained by diathermy loop excision allowed histopathologic examination. In experienced hands the diathermy loop does not cause significant artifacts that interfere with microscopic evaluation in the excised tissue. The pathologist was unable to make a diagnosis in only two of 424 cases, as a result of the complete absence of epithelium in the excised specimens. In all other cases a histologic diagnosis was established. All analyses were made without any revision of the histologic diagnosis. The final histopathologic diagnosis was compared with pretreatment cytologic diagnosis (Table II). In 178 of the cases studied (42%), the histologic diagnosis correlated entirely with pretreatment cytologic diagnosis. In 73 of 424 (17%) cases the final histopathologic diagnosis was more severe, and in 173 patients (41%) it was less severe than the initial pretreatment cytological diagnosis. Combining severe dysplasia and carcinoma in situ (grade 3 cervical intraepithelial neoplasia), the pretreatment cytologic diagnosis and histopathologic diagnosis corresponded in 73% of the cases. Smears were diagnosed as being suspicious for invasive cancer in 11 patients. Invasive cancer was confirmed histologically in only two of these patients. On the other hand, invasive carcinoma was histopathologically diagnosed in 14 patients, whereas the pretreatment cytologic diagnosis was not suspicious for invasive carcinoma. In 12 of these patients only microinvasive carcinoma was diagnosed.

For all patients, with the exception of five, diathermy loop excision unequivocally provided a definitive diagnosis on the basis of the most severe histopathologic abnormality present in that patient. Of the five patients

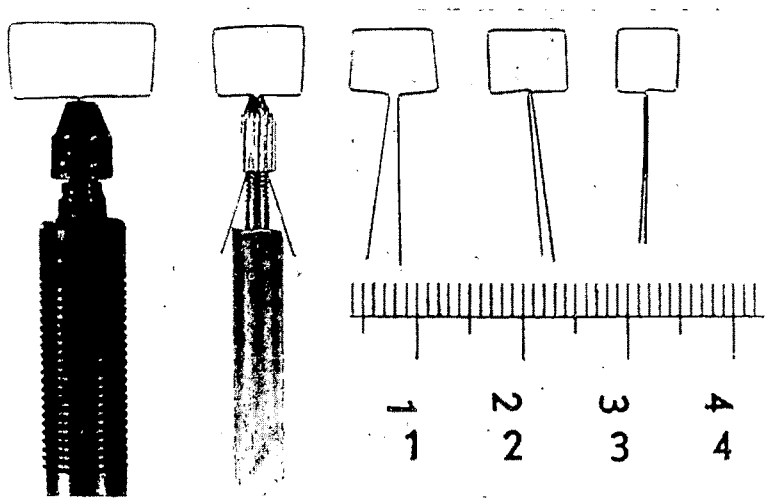


Fig. 1. Stainless steel diathermy loops as used in this study.

mentioned, the final histopathologic diagnosis in four cases was not grade 2 cervical intraepithelial neoplasia (as found with the initial diathermy loop excision) but grade 3 cervical intraepithelial neoplasia (as found with a second treatment procedure), and in one case the final histopathologic diagnosis was grade 2 instead of grade 1 cervical intraepithelial neoplasia (Table III). The diagnostic accuracy rate in this group was 99%.

Follow-up cervical smears were taken to evaluate the efficacy of the diathermy loop excision. On analysis of the first year of follow-up, 11 patients (2.6%) were lost to follow-up, 18 (4.2%) had undergone a second treatment before a first follow-up smear was taken, and 395 (93.2%) had one or more cytologic control examinations. In 285 of these 395 patients (72%) the first smear during follow-up was normal, and in 110 cases (28%) the first smear demonstrated cytologic findings consistent with mild dysplasia or a more severe lesion. Of these 110 patients with an abnormal first follow-up smear, 63 (57%) smears returned to normal within 1 year, without further intervention. When the first control smear was classified as consistent with mild dysplasia (n = 42), consecutive smears showed a return to normal in 88% of the patients in the first year. In the group of 285 patients showing no abnormalities in a first follow-up smear, subsequent smears showed abnormal findings in 25 patients. Cytologic findings were consistent with mild dysplasia in nine patients, moderate dysplasia in seven, and severe dysplasia carcinoma in situ in nine (Table IV).

The overall cure rate of primary diathermy loop excision, defined as the achievement of normal cytologic findings without further treatment, was 81% in this study. In 63 patients (15%) a second and in eight patients (2%) a third treatment procedure was performed after persistent abnormal cytologic findings. A diathermy loop excision was performed in 41 cases, conization in 10 cases, and hysterectomy in 20 cases (Table V). This means that 30 of 395 patients with cytologic control examinations had hysterectomy or conization as an ultimate treatment, resulting in an overall cure rate of 92% after diathermy loop excision in these patients.

Immediate and long-term morbidity. Hemorrhage can be a complicating factor during the healing process. In 32 cases (8%) secondary hemorrhage occurred during the first week after treatment. In 21 of these cases additional coagulation was necessary.

The healing process takes approximately 2 to 3 weeks. During this period, in which patients are discouraged from engaging in sexual intercourse, vaginal discharge is frequently seen. However, a manifest cervicitis after diathermy loop excision was seen in only one patient.

Stenosis of the cervical canal after primary diathermy loop excision was seen in four cases (1%). In the group of patients with a second diathermy loop excision a further case of stenosis was observed. Of these five patients, four were >45 years old.

Ninety-six patients tried to achieve pregnancy after diathermy loop excision and 78 succeeded. The total number of pregnancies in these patients was 96. Seventeen of these pregnancies resulted in abnormal labor (Table VI).

Infertility after diathermy loop excision was seen in 18 patients. Of these, 11 patients were already known to have fertility problems before treatment. Of the remaining seven patients, an unfavorable cervical milieu was part of the fertility problem in two patients. In these

Table II. Correlation between pretreatment cytologic findings and final histologic diagnosis after diathermy loop excision in 424 patients with cytologic findings consistent with cervical intraepithelial neoplasia

		Histologic diagnosis					
		No abnormalities		*Slight dysplasia*		*Moderate dysplasia*	
Cytologic diagnosis	*Total*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Slight dysplasia	20	5	25	7	35	3	15
Moderate dysplasia	72	9	12	10	14	25	35
Severe dysplasia	168	7	4	6	4	37	22
Carcinoma in situ	153	8	5	3	2	14	9
Suspicious for invasive carcinoma	11	—		—		—	
TOTAL	424	29		26		79	

Table III. Histologic features of primary diathermy loop excision compared with histologic outcome of subsequent treatments

	Histologic diagnosis at subsequent treatments					
Histologic diagnosis at primary diathermy loop excision	*Total*	*No abnormalities*	*Slight dysplasia (grade 1 cervical intraepithelial neoplasia)*	*Moderate dysplasia (grade 2 cervical intraepithelial neoplasia)*	*Severe dysplasia/carcinoma in situ (grade 3 cervical intraepithelial neoplasia)*	*(Micro)Invasive carcinoma*
No abnormalities	2	2	—	—	—	—
Slight dysplasia	2	—	1	1	—	—
Moderate dysplasia	15	3	6	2	4	—
Severe dysplasia/carcinoma in situ	67	18	4	10	25	—
(Micro)Invasive carcinoma	11	2	—	5	2	2

two patients cervical function was not known before treatment. An unfavorable cervical milieu was never the only fertility problem.

Comment

This study shows that diathermy loop excision is a combined diagnostic and therapeutic procedure for the management of patients with abnormal cervical cytologic findings. Patients in our study were selected on the basis of an abnormal smear. In the majority of the cases studied (58%), pretreatment cytologic findings did not correspond with the final histopathologic diagnosis. However, as has been reported by Van Nagell et al.[6] there was a good correlation between cytologic and histologic findings (73%) in this group with high-grade intraepithelial neoplasia (grade 3).

The management of cervical intraepithelial neoplasia lesions so far has involved many treatment modalities,[7-13] comprising excision techniques such as cold knife conization, electroconization, laserconization, diathermy loop excision, and hysterectomy or tissue destructive techniques such as cryocoagulation, electrocoagulation, and laser vaporization. In general, patients with cytologic findings suspicious for cervical intraepithelial neoplasia lesions will undergo colposcopy to confirm the abnormality of the epithelium and the extent of the lesion. Excision techniques can be performed after colposcopy without prior biopsy. When the transformation zone is not fully visible, many surgeons will opt for cold knife conization. The disadvantages of this technique are the need for hospital admission and general anesthesia.

Laser conization is a time-consuming procedure and the laser apparatus is very expensive. In addition, destructive techniques are restricted to patients with a satisfactory colposcopic examination, after exclusion of invasive carcinoma by prior biopsies.

Diathermy loop excision is a combined diagnostic and therapeutic procedure in which the entire transformation zone is removed. It can be performed with ease

Histologic diagnosis					
Severe dysplasia		*Carcinoma in situ*		*Microinvasive/invasive carcinoma*	
No.	%	*No.*	%	*No.*	%
4	20	1	5	—	
22	30	5	7	1	1
86	51	26	15	6	4
65	42	58	38	5	3
1	9	8	73	2	18
178		98		14	

Table IV. Analysis of follow-up cytologic results after diathermy loop excision: Cytologic findings in first follow-up smear compared with subsequent cytologic or histologic findings

	Subsequent cytologic and histologic diagnosis					
First follow-up smear	*Total*	*No abnormalities*	*Slight dysplasia*	*Moderate dysplasia*	*Severe dysplasia/carcinoma in situ*	*Lost to follow-up*
No abnormalities	285	243	9	7	9	17
Slight dysplasia	42	37	2	—	2	1
Moderate dysplasia	36	17	7	5	7	—
Severe dysplasia/carcinoma in situ	32	9	1	4	18	—

Table V. Second and third treatments after primary diathermy loop excision, performed in case of abnormal smear

	Second treatment	*Third treatment*
Hysterectomy	15	5
Conization	8	2
Diathermy loop excision	40	1
TOTAL	63	8

Table VI. Pregnancy outcome in 78 patients after diathermy loop excision

Total pregnancies	96
Term deliveries	79
Preterm deliveries (>28 but <38 wks)	6
Immature delivery (<28 wk)	1
Abortion	8
Prenatal death	1
Cesarean section (failure to progress)	1

in an outpatient clinic in the same session as colposcopy. It takes only about 1 to 2 minutes for the excision and 2 to 3 minutes to obtain hemostasis, and in addition the equipment is inexpensive.

Histopathologic examination of the tissue encompassing the entire transformation zone avoids missing or undergrading cervical intraepithelial neoplasia lesions, and (micro)invasive carcinoma. Pathologists raise the objection that the completeness of treatment cannot be assessed because of the loss of defined tissue margins. However, to evaluate the completeness of treatment, the finding of a cytologic abnormality has proved to be a more useful prognostic indicator than the histologic examination of excision margins.[14] Residual disease with crypt involvement, situated at the surface after healing, is probably due to the open excision margins as a consequence of the diathermy loop excision technique. Therefore residual disease is easily detected by follow-up cytologic testing. Furthermore, in contrast to cold knife conization or cryocoagulation, residual disease will not be hidden by cicatrization.

The excellent cervical morphologic features after diathermy loop excision, with optimal localization of the neosquamocolumnar junction, (NSCJ) permit good

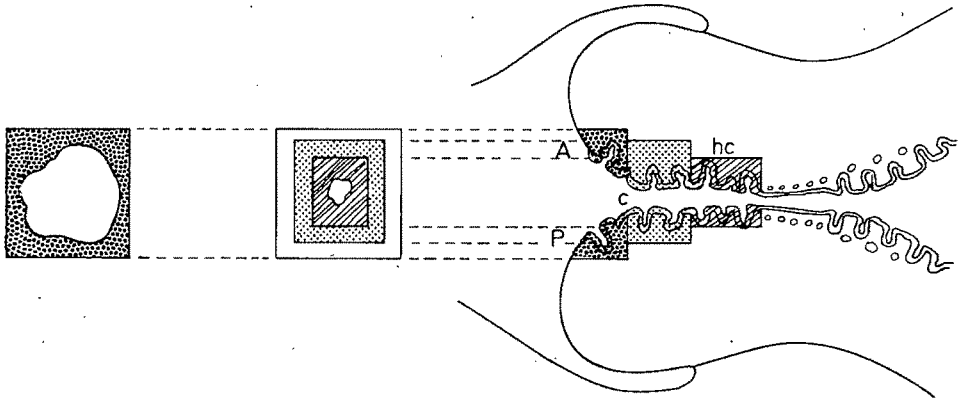


Fig. 2. Schematic sectional view of cervix uteri. *A*, Anterior lip; *P*, posterior lip; *c*, central; *hc*, high central.

cytologic follow-up. The follow-up smear should always contain a combination of columnar and squamous epithelial cells. A control smear that contains only squamous epithelial cells is inadequate, and the smear must be repeated. The fact that 63 of 110 (57%) abnormal first follow-up smears returned to normal at subsequent cytologic examination without additional intervention justifies an expectant approach to low-grade cervical intraepithelial neoplasia diagnosed in follow-up smears. When cytologic findings consistent with severe dysplasia or carcinoma in situ (grade 3 cervical intraepithelial neoplasia) occur in follow-up smears, then further investigation is necessary.

The overall success rate of diathermy loop excision in this study was 92%. This result was in the same range as that obtained with both destructive techniques, cryocoagulation and laser vaporization.[9-11] However, these destructive techniques are restricted to patients without endocervical extension of the lesion, a group of patients that has the lowest risk of residual disease. Furthermore, the success rate was found to be in the same range as that obtained with laser conization.[10, 11] Compared with the laser technique, diathermy loop excision is less time-consuming, cheaper, and much easier to perform.

In this study we used rectangular loops. The advantage of this loop is that the control of the depth of the excision is much better. The actual excision itself, however, may be insufficient. Excision margins should extend 3 to 5 mm beyond the margins of the transformation zone, both on the ectocervix and in the endocervical canal. When the lesion extends into the endocervical canal and is not entirely visible, we recommend the removal of an additional deeper central part (Fig. 2). Because diathermy loops in various forms are commercially available, many surgeons may choose a semicircular loop and perform a conelike excision. Even so, when a semicircular loop is used, the excision of an additional central part to prevent undertreatment is advisable.

Hemorrhage is a well-known complication of cold knife conization. Before coagulation was introduced, sutures were used to stop bleeding. Nowadays the use of hemostatic sutures in excision techniques is rare. To perform laser conization, local anesthesia in combination with a vasoconstrictor is necessary. With a diathermy loop, hemostasis can be well controlled even without vasopressin. In 8% of patients secondary hemorrhage was seen during the healing process. In most of the cases additional coagulation was necessary. Secondary hemorrhage is a well-known complication of cold knife conization, but with laser conization secondary hemorrhage is very rare.[10, 11]

An increasing number of women of reproductive age are being subjected to a local treatment procedure that may alter the cervical environment and, in so doing, could theoretically affect cervical competence. Our results demonstrate that diathermy loop excision does not affect fertility and pregnancy outcome. However, individual exceptions may be caused by extreme scarring or reduction of cervical mass. This agrees with the results obtained with other treatment modalities, as reported by others.[9, 10, 13, 15, 16]

The results of this large series of patients warranted the conclusion that diathermy loop excision offers a good alternative in the management of cervical intraepithelial neoplasia lesions. It has all the advantages of an outpatient treatment, is just as effective as all other techniques, and requires much less expensive equipment. The excellent anatomic status of the cervix after diathermy loop excision permits good cytologic follow-up, which has been shown to be sufficiently reliable to detect residual disease.

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Fetal polycythemia and thrombocytopenia in pregnancies complicated by maternal diabetes mellitus

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In 40 pregnancies complicated by maternal diabetes mellitus umbilical venous blood was obtained by cordocentesis within 24 hours of elective delivery at 36 to 40 weeks' gestation. The mean fetal hematocrit was significantly higher and the mean platelet count significantly lower than the corresponding values of our reference ranges. Furthermore, blood gas analysis demonstrated these fetuses to be normoxemic but acidemic. The degree of fetal acidemia was significantly associated with both maternal and fetal blood glucose concentrations. The fetal hematologic indices were significantly related to the maternal glycosylated hemoglobin percentage but not to the degree of fetal acidemia or to the maternal or fetal blood glucose concentration at the time of cordocentesis. Fetal acidemia, polycythemia, and thrombocytopenia may contribute to the increased incidence of late unexplained intrauterine deaths in pregnancies complicated by maternal diabetes mellitus. (Am J Obstet Gynecol 1992;166:1287-92.)

Key words: Diabetes mellitus, cordocentesis, fetal hematology, blood gases, glycosylated hemoglobin

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6/1/34059

There is a well-recognized association between maternal diabetes mellitus and late unexplained fetal death.[1] Although the cause remains uncertain, there is indirect evidence implicating fetal hypoxia. Thus in diabetic pregnancies the incidence of abnormal fetal heart rate patterns in labor and low Apgar scores at delivery is increased.[2, 3] Furthermore, analysis of cord blood at delivery has demonstrated decreased Po_2 and

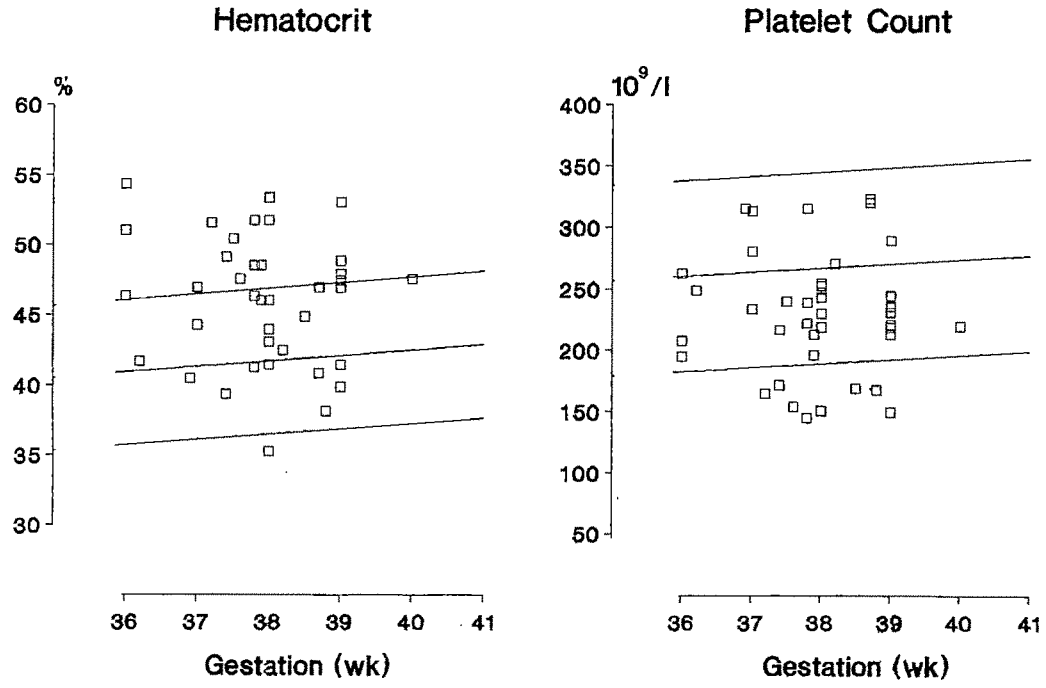


Fig. 1. Hematocrit and platelet count plotted on appropriate reference range (mean with 95th and 5th percentiles) with gestation.

pH and increased erythropoietin concentration in association with neonatal polycythemia.[4-6] More recently Bradley et al.[7] measured fetal blood gases at cordocentesis in pregnancies complicated by maternal diabetes mellitus and reported that the mean pH was significantly lower than in nondiabetic controls.

The aim of the current study was to investigate the relationship between possible hematologic changes in fetuses of diabetic mothers and maternal diabetic control and fetal blood gases. Fetal blood samples were obtained by cordocentesis rather than at delivery because the process of birth itself may influence the measured parameters.

Patients and methods

Umbilical venous blood was obtained by cordocentesis up to 24 hours before delivery at 36 to 40 weeks' gestation in 40 women with established (n = 32) or gestational (n = 8) diabetes. A maternal venous blood sample was also taken from the antecubital fossa before cordocentesis. In each case gestational age was established from the menstrual history (n = 32) or by an early ultrasonographic scan in those with uncertain dates (n = 8). The patients were recruited from our diabetic antenatal clinic. Before giving their written consent, the patients were counseled that the procedure was experimental and that the results would not give any direct benefit to their current pregnancy. The study was approved by our hospital ethics committee.

All patients were insulin treated, and according to the White classification 23 belonged to group B, 15 to group C, and two to group D.[8] None of the patients had chronic hypertension or preeclampsia. The pregnancies were managed expectantly; according to our current policy and in consultation with individual patients, elective delivery was undertaken at 36 to 40 weeks' gestation, depending on fetal growth and well-being and previous obstetric and medical history. In 24 cases the mode of delivery was by elective cesarean section for previous cesarean section (n = 12), suspected macrosomia (n = 5), breech presentation (n = 4), congenital abnormality of the maternal pelvis (n = 1), poor obstetric history (n = 1), and maternal ischemic heart disease (n = 1). Induction of labor was undertaken in 16 patients; 11 had vaginal deliveries and five had emergency cesarean section because of suspected cephalopelvic disproportion (n = 1) or abnormal intrapartum fetal heart rate patterns (n = 4).

Cordocentesis was performed without fetal paralysis or maternal fasting or sedation, and all procedures were uncomplicated.[9] Fetal blood was collected into a heparinized syringe (350 μl) for blood gas analysis (Radiometer ABL330, Copenhagen) and for measurement of whole blood glucose (YSI 23A, Yellow Springs Instruments Co., Yellow Springs, Ohio). Fetal blood was also collected in tubes containing ethylenediametetraacetic acid (500 μl) for measurement of hematocrit and platelet and total white blood cell counts (Coulter

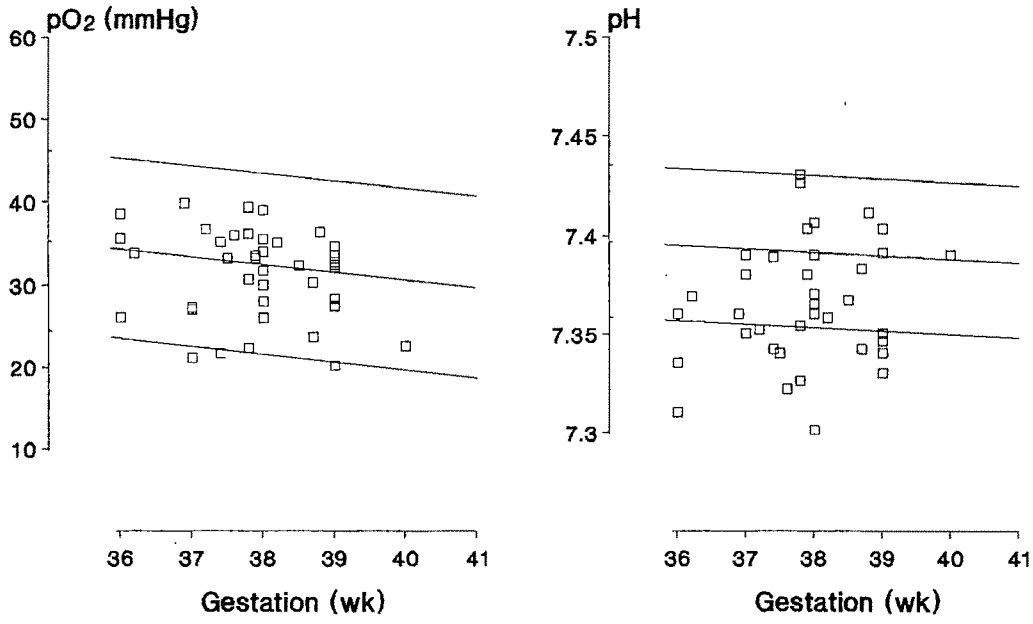


Fig. 2. Umbilical venous blood Po_2 and pH plotted on appropriate reference range (mean with 95th and 5th percentiles) with gestation.

S-Plus, Coulter Electronics, Luton, United Kingdom). Kleihauer testing confirmed that all samples contained only fetal blood. Blood films were stained with Jenner's giemsa on an automatic processing machine, and the corrected white blood cell count and number of erythroblasts per 100 white blood cells were determined. At the time of cordocentesis a maternal blood sample was taken from the antecubital fossa for the measurement of whole blood glucose concentration as described and the glycosylated hemoglobin percentage by electroimmunodiffusion (Corning scanner, Corning, Halstead, United Kingdom).

Umbilical venous blood was also collected at delivery from 26 of the patients; there were 14 elective cesarean sections with patients under epidural ($n = 7$) or general ($n = 7$) anesthesia, five emergency cesarean sections, and seven vaginal deliveries. The cord was double clamped immediately after delivery, and an umbilical venous blood sample was taken in heparinized syringes for measurement of pH.

Unpaired Student t tests were applied to investigate if the measured parameters differed from the reference ranges for normal pregnancies. For birth weight and maternal glycosylated hemoglobin percentage, published ranges were used.[9, 10] For the others our reference ranges with gestation were used: hematocrit 2.425 + 0.396 × weeks, SD 0.0211, $n = 311$; platelet count 129.5 + 3.62 × weeks, SD 46.74, $n = 311$; corrected white blood cell count 0.152 + 0.104 × weeks − 0.004 × weeks^2 + 0.0005 × weeks^3, SD 0.0283, $n = 311$; umbilical venous blood pH 7.47 − 0.0023 × weeks, SD 0.0306, $n = 268$; and umbilical venous blood Po_2 62.305 − 0.811 × weeks, SD 7.512, $n = 268$. The patient selection and methods used to establish these ranges were as previously detailed.[11-13] By comparison with the normal ranges, all hematologic parameters, blood gas values and birth weights for the diabetic pregnancies were expressed as the number of standard deviations by which individual values differed from the normal mean for gestation (Δ-value). Regression analysis was then used to define any significant associations between hematologic Δ-values and blood gas Δ-values, birth weight Δ-values, maternal glycosylated hemoglobin percentage, and fetal and maternal blood glucose concentrations. Regression analysis was also used to define the significance of associations in blood gas data at cordocentesis and delivery.

Results

In the 40 diabetic pregnancies the mean fetal hematocrit was significantly higher than the normal mean for gestation (Fig. 1; mean difference 1.014 SD, SE 0.236, $t = 4.29$, $p < 0.0001$). In contrast, mean platelet count was significantly lower (Fig. 1; mean difference −0.802 SD, SE 0.168, $t = -4.77$, $p < 0.0001$). The mean white blood cell count was not significantly different (mean difference −0.273 SD, SE −0.271, $t = -1.01$).

The fetal blood pH was significantly lower (Fig. 2; mean difference −1.087 SD, SE 0.192, $t = -5.67$, $p < 0.0001$), whereas the Po_2 was not significantly different from the appropriate normal mean for gestation

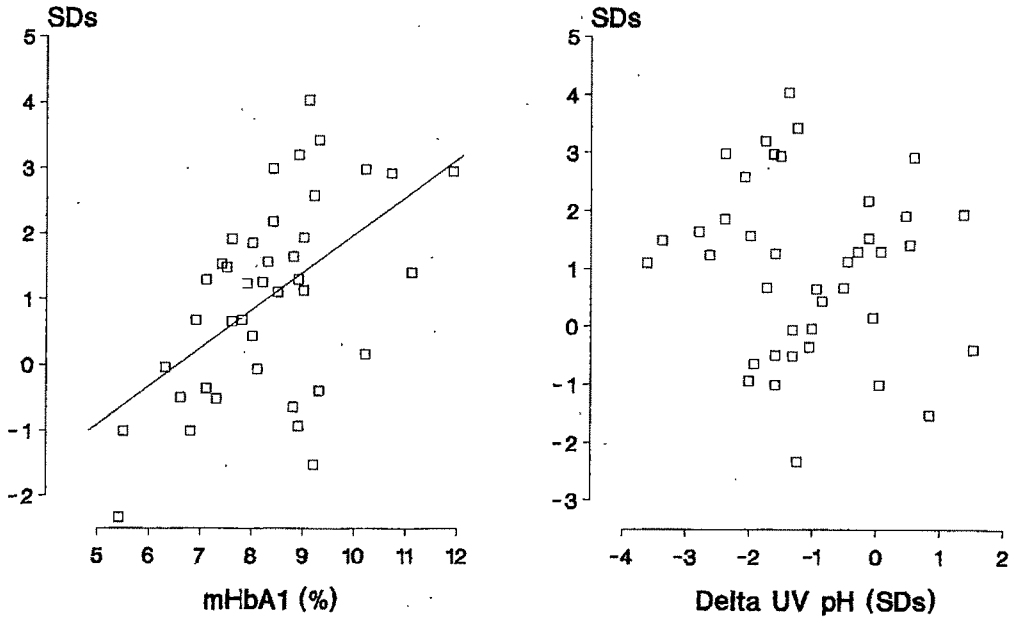


Fig. 3. Relationship between Δ-value of fetal hematocrit and maternal glycosylated hemoglobin percentage *(mHbA$_1$)* and between Δ-value of fetal hematocrit and Δ-value of umbilical venous pH *(Delta UV pH)*.

Table I. Association between fetal hematocrit, platelet count, blood gas values (pH and Po_2), fetal umbilical venous glucose concentration, fetal birth weight, and maternal glycosylated hemoglobin percentage

	Δ-Value of hematocrit (r)	*Δ-Value of platelet count (r)*
Δ-Value of platelet count	−0.358*	
Δ-Value of pH	−0.133	−0.132
Δ-Value of Po_2	−0.032	−0.285
Δ-Value of birth weight	0.171	0.015
Umbilical venous glucose concentration	0.091	−0.002
Maternal glycosylated hemoglobin	0.537†	−0.348*

All values except maternal glycosylated hemoglobin and umbilical venous glucose concentration were expressed as difference from normal mean for gestation in standard deviations (Δ-values).

*$p < 0.05$.

†$p < 0.001$.

(Fig. 2; mean difference −0.201 SD, SE 0.128, $t = -1.57$). The maternal glycosylated hemoglobin percentage and birth weight were significantly higher than the appropriate normal means for gestation (mean difference 1.382 SD, SE 0.309, $t = 6.10$, $p < 0.0001$ and mean difference 1.007 SD, SE 0.206, $t = 4.89$, $p < 0.0001$, respectively).

The associations between Δ-values for fetal hematologic findings and those for fetal blood gases, fetal blood glucose concentration, fetal size, and maternal glycosylated hemoglobin are shown in Table I. There were significant associations between Δ-values for fetal hematocrit and platelet count. In addition, Δ-values for both fetal hematocrit (Fig. 3) and platelet count (Fig. 4) were significantly associated with maternal glycosylated hemoglobin but not with fetal blood gas values, fetal glucose concentration, or Δ-value for fetal weight. There was a significant association between both the maternal and the fetal blood glucose concentration and Δ-value for fetal pH ($r = -0.449$, $p < 0.01$ and $r = -0.397$, $p = 0<0.05$, respectively).

In the 26 patients in whom umbilical venous blood pH was measured both at cordocentesis and at delivery there was no significant association between the predelivery and postdelivery data ($r = 0.15$). In all but one case the pH after delivery was lower than the pH at cordocentesis, and the greatest decrease was in those patients with vaginal deliveries (Fig. 5).

All infants were healthy at birth and normal anatomy was confirmed. There was no clinical evidence of any thromboses in the neonatal period.

Comment

This study has demonstrated that in pregnancies complicated by maternal diabetes mellitus some fetuses are acidemic, polycythemic, thrombocytopenic, and macrosomic. Furthermore, there are significant asso-

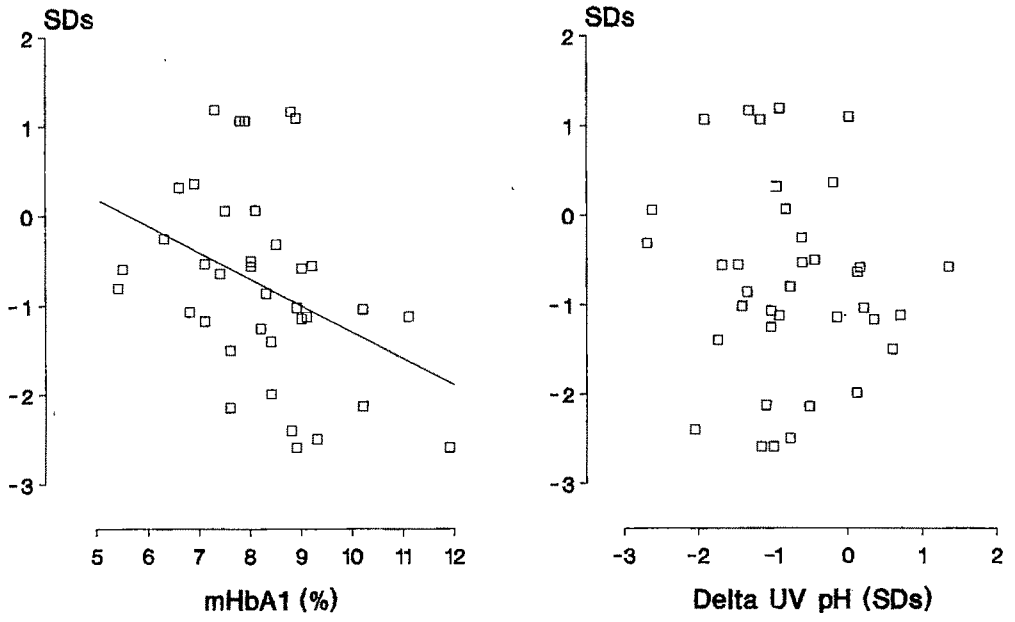


Fig. 4. Relationship between Δ-value of fetal platelet count and maternal glycosylated hemoglobin percentage *(mHbA$_1$)*, and between Δ-value of fetal platelet count and Δ-value of umbilical venous pH *(Delta UV pH)*.

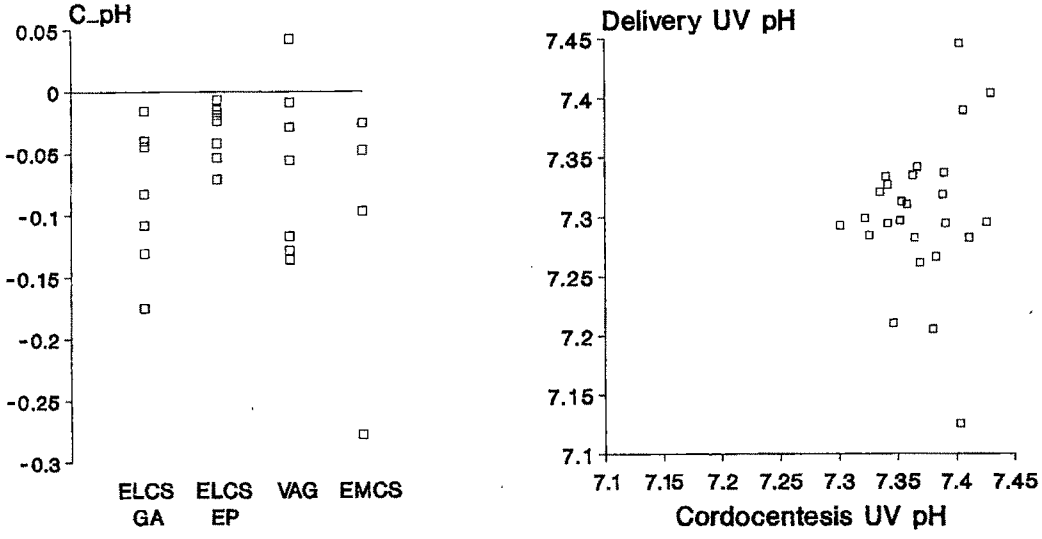


Fig. 5. Change in umbilical venous blood pH *(C pH)* between cordocentesis and delivery in 26 patients *(left)*, according to mode of delivery; elective cesarean section with patients under general anesthetic *(ELCS GA)*, elective cesarean section with patients under epidural anesthetic *(ELCS EP)*, vaginal delivery *(VAG)*, and emergency cesarean section *(EMCS)*. Relationship between umbilical venous blood pH *(UV pH)* at cordocentesis and delivery *(right)*.

ciations between the degree of polycythemia and thrombocytopenia and maternal glycemic control, as reflected in increased maternal glycosylated hemoglobin percentage. The lack of a significant association in the umbilical venous blood pH between results at cordocentesis and those at delivery demonstrates that fetal biochemistry may be altered by the process of birth.

The finding of acidemia in a substantial proportion of the fetuses provides support for the concept that altered acid-base status may contribute to the increased

incidence of late unexplained stillbirths in pregnancies complicated by maternal diabetes. The significant association between both maternal and fetal glucose concentrations and the degree of fetal acidemia suggests that the latter may be the consequence of increased metabolic rate, which is a result of fetal hyperglycemia. The data provide an extension to the previous observations of Bradley et al.,[7] who reported that fetal acidemia was not associated with hypoxemia. Chronic hyperglycemia in pregnant sheep results in increased fetal glycolysis, accumulation of lactate, and fall in pH.[14, 15] Fetal anaerobic glycolysis may occur in the presence of hyperglycemia, because animal studies have shown there is reduced capacity for oxidative metabolism and gluconeogenesis and low pyruvate dehydrogenase activity in intrauterine life.[16] Furthermore, minor elevations in fetal blood glucose are associated with acidemia in the absence of hypoxemia.[15]

In the neonatal period polycythemia is most commonly defined as a hematocrit >65%, and the reported incidence in infants of diabetic mothers is 16% to 34%.[6, 17] This wide range may be partly a consequence of the different methods used in these studies, including gestational age, mode of delivery, time of cord clamping, and the site and time of sampling. In our study polycythemia is defined as a hematocrit >95th percentile for gestational age, and the incidence was 40%. The significant association of fetal hematocrit with maternal glycosylated hemoglobin percentage, but not with fetal or maternal glucose concentration at cordocentesis, implies that long-term rather than short-term glycemic control is important in the development of polycythemia. Pedersen's theory[18] suggests that maternal hyperglycemia causes fetal hyperglycemia and hyperinsulinemia. Insulin may stimulate erythropoiesis either through a direct effect on the marrow[19] or indirectly through increased metabolic rate, tissue hypoxia, and consequent erythropoietin release.

Fetal thrombocytopenia, like polycythemia, was associated with maternal glycosylated hemoglobin percentage but not with maternal or fetal blood glucose concentration at the time of cordocentesis. The most likely cause of the mild fetal thrombocytopenia is increased platelet consumption, because previous studies have demonstrated that in infants of diabetic mothers there is increased platelet aggregation.[20] The abnormalities in infants of diabetic mothers include increased platelet endoperoxide formation and decreased vascular prostacyclin production.[20, 21]

The combination of fetal polycythemia (with consequent hyperviscocity) and increased platelet aggregation could offer an explanation for the increased incidence of intravascular thromboses in infants of diabetic mothers.[22, 23] Indeed, it could lead to intrauterine thrombotic episodes, offering an alternative to acidemia as the underlying cause for late unexplained intrauterine deaths. In a postmortem study of 4000 neonatal deaths the incidence of venous thromboses in infants of diabetic mothers was 15.8% compared with an incidence of <1% in the remaining cases.[23] Furthermore in the infants of diabetic mothers the thrombi showed more frequent organization and calcification, suggesting intrauterine formation.

In pregnancies complicated by maternal diabetes the incidence of unexplained stillbirths has been reduced with intense multidisciplinary antenatal care; however, it remains a well-recognized complication of diabetic pregnancy.[24] Fetal acidemia and increased tendency for intravascular thromboses, either individually or in combination, could be the underlying cause.

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Actocardiotocographic monitoring of triplets during vaginal delivery

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A case is reported in which fetal monitoring during vaginal delivery of triplets was performed with the MT 430 actocardiotocograph. This method provides optimal monitoring of multiple pregnancies because the fetal heart rate tracings of three fetuses, together with recordings of fetal movements and uterine contractions, can be visualized on one display. (AM J OBSTET GYNECOL 1992;166:1293-4.)

Key words: Actocardiotocograph, triplets, fetal monitoring, delivery

The numbers of multiple births have increased dramatically in many countries as a result of the widespread use of ovulation-inducing agents and assisted reproductive technologies. For example, in the United States the number of twins and triplets—higher-order births increased by 33% and 101%, respectively, during the decade between 1978 and 1988. Similar increases have been observed in the United Kingdom and Germany.

At present, the mode of delivery of triplets is controversial. If the vaginal route is chosen, along with other requirements it is mandatory to obtain optimal monitoring of all three fetuses. In 1984 Maeda[1] described the actocardiotocograph, which permits registration of the fetal heart rate (FHR) tracing in combination with fetal movements and uterine contractions.[1] Recently an advanced model, the MT430 actocardiotocograph (Toitu Co., Ltd., Tokyo, Japan), was developed for use with multiple pregnancies, and first attempts were made to define fetal behavior in twins.[2]

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6/1/35279

Case report

A 30-year-old woman, para 1, became pregnant with triplets after in vitro fertilization. At 14 weeks' gestation elective cerclage was performed. At 26 weeks the patient was admitted for bed rest because of increasing mechanical discomfort. Periodic ultrasonographic examinations revealed three fetuses, in vertex position, having normal growth. External FHR tracings, and Doppler flow measurements of the umbilical artery were normal in all three fetuses. At 35 weeks the cerclage suture was removed, after which labor began. After artificial rupture of the membranes of the first amniotic sac, a scalp electrode was placed on the fetal head, followed by insertion of a catheter for measurement of intrauterine pressure. The second and third fetuses were externally monitored with respect to FHR and fetal movement. Five hours after amniorrhexis full dilatation was present. Shortly thereafter the three infants were born in vertex position during a 15-minute period. Throughout labor and delivery, FHR tracings of all three fetuses, together with recordings of uterine contractions and fetal movement of the two externally

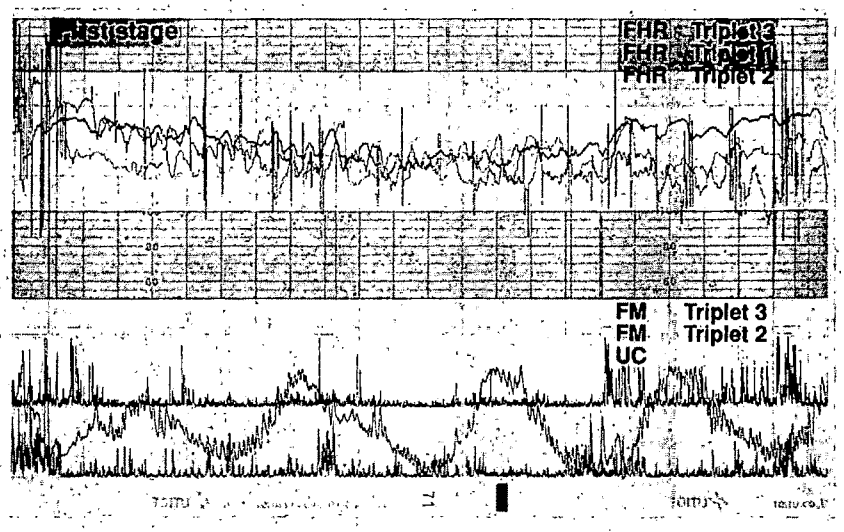


Fig. 1. Actocardiotocographic tracing of triplets during delivery. *FM*, Fetal movement; *UC*, uterine contraction.

monitored fetuses, were visualized on one display panel (Fig. 1). Birth weights of the infants were 2000, 2180, and 1680 gm, respectively, and umbilical cord pH was 7.35, 7.31, and 7.39, respectively.

Comment

Fetal monitoring of triplets generally is a time-consuming effort, necessitating the use of a variety of equipment. When several machines are used or recordings are performed in sequence, there is a risk of monitoring one or more fetuses twice, leaving one or even two fetuses unexamined. The MT430 actocardiotocograph is a small, portable device capable of collecting and displaying all FHR tracings simultaneously along with the intensity of uterine contractions and the movement patterns of two of the three fetuses.

The two external FHR and fetal movement tracings are registered by only two separate transducers with a carrier frequency of 1.1 MHz and an intensity of <1.1 mW/cm^2. During vaginal delivery of triplets, the third FHR tracing is internal. Uterine contractions can be monitored either externally or internally. The FHR is documented according to the principle of autocorrelation at a range of 50 to 240 beats/min. Fetal movements are documented simultaneously by the same transducer in an analog manner. Thus the density of the spikes reflects the frequency of fetal movement, and the amplitude reflects the velocity of fetal movement.

The use of the MT430 actocardiotocograph provides optimal fetal monitoring during vaginal delivery of triplets.

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Abnormal 1,25-dihydroxyvitamin D metabolism in preeclampsia

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We previously reported that preeclampsia is associated with hypocalciuria (N Engl J Med 1987;316:715). The purpose of this study was to determine whether alterations in calcium regulatory hormones are present in preeclampsia and, if so, whether they are responsible for hypocalciuria. Thirty-two pregnant women were studied in the second and third trimesters of pregnancy (11 women with preeclampsia, nine with chronic hypertension, and 12 normotensive women). 1,25-Dihydroxyvitamin D, C-terminal parathyroid hormone, ionized calcium, and urinary calcium excretion were measured. 1,25-Dihydroxyvitamin D was significantly lower in the women with preeclampsia in the third trimester when the disease developed (37.8 ± 15 pg/ml) than in women with chronic hypertension (75 ± 15 pg/ml, $p < 0.05$) and normal women (65 ± 10 pg/ml, $p < 0.05$). Parathyroid hormone was higher, but not significantly, in those with preeclampsia. Ionized calcium was not significantly different among the three groups. Urinary calcium excretion was abnormally low for pregnancy (<50 mg/24 hr) in all but one women with preeclampsia. We conclude that 1,25-dihydroxyvitamin D is reduced in preeclampsia and may lead to hypocalciuria by causing decreased intestinal absorption of calcium, stimulation of parathyroid hormone, and increased distal renal tubular resorption of calcium. The cause of reduced 1,25-dihydroxyvitamin D in preeclampsia is unknown and may be due to either diminished renal or placental production of the hormone. (Am J Obstet Gynecol 1992;166:1295-9.)

Key words: Preeclampsia, calcium metabolism, 1,25-dihydroxyvitamin D, hypocalciuria

Alterations in calcium metabolism have been reported in human essential hypertension and experimental animal models of hypertension and have been implicated in the pathogenesis of elevated blood pressure.[1-3] It has also been suggested recently that calcium regulation may be abnormal in preeclampsia, a hypertensive disorder unique to pregnancy.

We previously reported that preeclampsia is associated with hypocalciuria,[4] an observation subsequently confirmed by other investigators.[5-7] Moreover, we suggested that measurement of urinary excretion of calcium may be useful in distinguishing preeclampsia from other forms of gestational hypertension. We hypothesized that hypocalciuria is due to increased renal tubular resorption of calcium rather than decreased filtered load, because fractional excretion of calcium was significantly lower in women with preeclampsia.

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We report here an investigation of the hormonal basis for hypocalciuria. We determined the concentrations of 1,25-dihydroxyvitamin D, parathyroid hormone, and serum and urinary calcium in women with preeclampsia and compared them with those of normotensive pregnant women and women with chronic hypertension. It was considered important to study 1,25-dihydroxyvitamin D levels because either a low level (e.g., primary deficiency leading to decreased serum calcium, increased parathyroid hormone, and increased renal tubular resorption of calcium) or a high level (e.g., primary calcium deficiency leading to increased parathyroid hormone, increased tubular resorption of calcium, and increased renal 1α-hydroxylase activity) might be associated with hypocalciuria, and possible therapeutic options might be different.

We report here that 1,25-dihydroxyvitamin D levels are significantly lower in preeclampsia and parathyroid hormone is higher, compared with levels in normal and chronically hypertensive pregnant women.

Methods

Thirty-two pregnant women were studied during the second and third trimesters of pregnancy. Preeclamptic women were recruited from consecutively hospitalized patients with the disease. An attempt was made to minimize the uncertainty in the diagnosis of preeclampsia;

Table I. Clinical characteristics of study patients (third trimester)

	Normal (n = 9)	*Chronic hypertension (n = 9)*	*Preeclampsia (n = 9)*
Maternal age (yr)	29 ± 4.2	32 ± 2.8	26 ± 6*
Gestational age (wk)	33.9 ± 2.7	35 ± 0.8	31.2 ± 3.7
Parity			
Nulliparous	8	4	7
Multiparous	1	5	2
Race			
Black	0	2	4
White	7	7	4
Other	2	0	1

*$p < 0.02$, compared with chronic hypertension.

Table II. Blood pressure and laboratory data on study patients (third trimester)

	Normal (n = 9)	*Chronic hypertension (n = 9)*	*Preeclampsia (n = 9)*
Maximal mean arterial pressure (mm Hg)	83 ± 5	102 ± 10*	131 ± 17†
Urine protein (mg/24 hr)	93 ± 47	181 ± 56	3631 ± 4114‡
Serum uric acid (mg/dl)	5.3 ± 1.07	4.03 ± 1.06	8.4 ± 1.5§
Serum creatinine (mg/dl)	0.8 ± 0.06	0.7 ± 0.11	1.1 ± 0.39‡
Platelet count ($\times 10^5$)	250 ± 80	244 ± 64	144 ± 115

Mean arterial pressure calculated as: (Systolic pressure plus 2 × Diastolic pressure) divided by 3.

*$p < 0.02$, compared with normal pregnancy.

†$p < 0.001$, compared with normal pregnancy and chronic hypertension.

‡$p < 0.05$, compared with chronic hypertension.

§$p < 0.01$, compared with normal pregnancy and chronic hypertension.

therefore patients with mild disease were excluded. Women with chronic hypertension were recruited from the office practices of the investigators. Normotensive women were healthy pregnant volunteers recruited from the employees of The New York Hospital. Patients with preexisting intrinsic renal disease were excluded. None of the subjects was receiving diuretics during the study. All patients were taking prenatal vitamins that contained 200 to 600 mg of calcium carbonate per tablet. All patients were eating an unrestricted diet either at home or, in the case of the preeclamptic women, in the hospital. Hypertension in pregnancy was defined as the presence of a blood pressure of 140/90 mm Hg or a rise of 30 mm Hg in systolic pressure or 15 mm Hg in diastolic pressure. Preeclampsia was defined as hypertension associated with excretion of >300 mg of urinary protein per 24 hours (or >2+ in a single voided specimen) or abnormal liver function tests and decreased platelet count.[8] Chronic hypertension was defined as the presence of a documented blood pressure of >140/90 mm Hg before pregnancy. None of the patients with chronic hypertension had superimposed preeclampsia.

Venous blood was collected from pregnant women in the second and third trimesters. Twenty-four–hour urine collections were obtained at the same times, except when a woman with severe preeclampsia was delivered <24 hours after admission to the hospital. Serum ionized calcium was measured with a calcium ion electrode apparatus in serum collected under anaerobic conditions. 1,25-Dihydroxyvitamin D was measured after chromatography on Sep-Pak C_{18} columns by a microassay with receptors from calf thymus.[9] Parathyroid hormone was measured by a radioimmunoassay with an antibody that recognizes the C-terminal fragment (Smith Kline & French, Van Nuys, Calif.). Serum levels of uric acid and creatinine were assayed by multichannel automated techniques. Urinary creatinine levels were determined by an autoanalyzer, and urinary calcium was measured by atomic absorption spectroscopy. Urinary protein was measured by nephelometry after precipitation with trichloroacetic acid.[10]

Mean arterial pressure was calculated as follows: (Systolic pressure + 2 × Diastolic pressure) divided by 3. Analysis of variance and the Student–Neuman-Keuls tests were used to determine differences between normal, chronic hypertensive, and preeclamptic women. All p values are two-tailed.

Results

The study population ($n = 32$) consisted of 11 women with preeclampsia (two were delivered in the second trimester, nine in the third), nine women with chronic hypertension (all were studied in both second

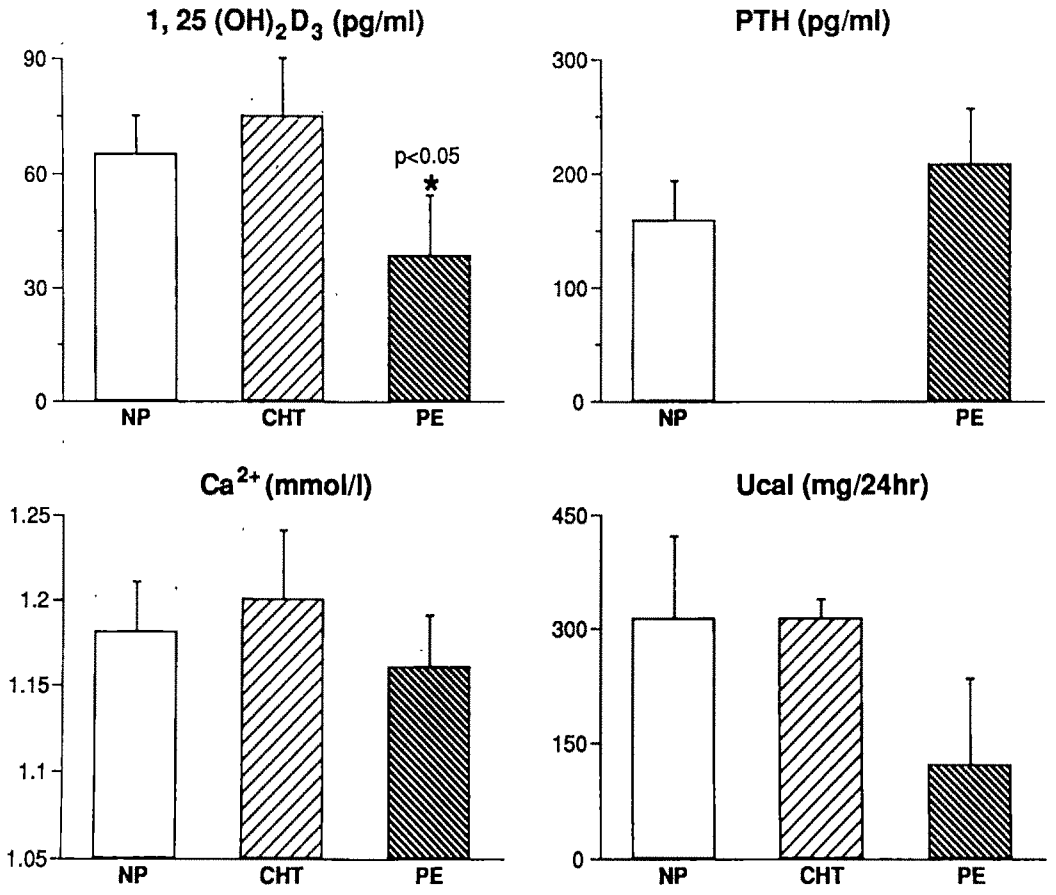


Fig. 1. Mean ± SD 1,25-dihydroxyvitamin D_3 *(1,25 [OH]$_2$ D_3)* C-terminal parathyroid hormone *(PTH)*, serum ionized calcium *(Ca^{2+})*, and urine calcium excretion *(U_{Cal})* in third-trimester pregnant women. *NP*, Normotensive women (n = 9); *CHT*, chronic hypertensive women (n = 9); *PE*, preeclamptic women (n = 9).

and third trimesters), and 12 normotensive women (three were studied in the second trimester alone, three in both second and third trimesters, and six only in the third trimester). It was considered important to present the data for patients studied at a similar gestational age; therefore the data obtained from patients in the third trimester were analyzed separately from those obtained in the second trimester. Because of the smaller number of patients with preeclampsia in the second trimester, only data from third-trimester patients are presented in the tables and figures.

The clinical characteristics of the third-trimester patients are shown in Table I. Two of the nine preeclamptic women were multiparous but had new mates for the pregnancies in this study. As shown in Table II, the preeclamptic women had significant hypertension, proteinuria, hyperuricemia, azotemia, and low platelet counts.

Fig. 1 compares 1,25-dihydroxyvitamin D, parathyroid hormone, and serum and urinary calcium concentrations found in preeclamptic, normotensive, and chronic hypertensive pregnancies in the third trimester. 1,25-Dihydroxyvitamin D levels were significantly lower in women with preeclampsia when compared with either chronic hypertensive or normotensive women. The mean ± SD concentration in the nine preeclamptic women studied in the third trimester was 37.8 ± 15.5 pg/ml, compared with 75 ± 15 pg/ml in the chronic hypertensive, and 65 ± 10 pg/ml in the normal pregnant women ($p < 0.05$). Parathyroid hormone levels tended to be higher in women with preeclampsia than in normotensive women; however, the differences did not reach statistical significance. One woman with severe preeclampsia, hypocalciuria, and low levels of 1,25-dihydroxyvitamin D had an extraordinarily high level of parathyroid hormone (132,000 pg/ml), which normalized postpartum. The basis for this marked pregnancy-related increase is not clear. It is possible that altered renal function, which is present in preeclampsia, contributed to diminished clearance of the carboxyterminal fragment of the parathyroid hormone molecule. This patient was excluded from the

statistical analysis of the parathyroid hormone levels. Urinary calcium excretion was abnormally low (<50 mg/24 hr) in all except one preeclamptic woman in whom 24-hour urine samples were obtained.

The two preeclamptic women who had severe disease and were delivered in the second trimester also had lower 1,25-dihydroxyvitamin D levels (21 and 41 pg/ml). The mean ± SD was 62 ± 27 pg/ml in the six second trimester normal women and 59 ± 8 pg/ml in the nine chronic hypertensive women studied at a similar gestational age. Thus these second-trimester preeclamptic women clearly had abnormally low 1,25-dihydroxyvitamin D levels. Parathyroid hormone was elevated (350 pg/ml) in one of the two women. Urine calcium excretion could only be measured in one (the other was delivered immediately), and it was extremely low (34 mg/24 hr), compared with 234 ± 43 mg/24 hr in normotensive second-trimester women and 201 ± 24 mg/24 hr in women with chronic hypertension.

Although several patients with preeclampsia had low ionized calcium levels, they were not significantly different from the other groups. One woman with severe preeclampsia had a markedly decreased creatinine clearance (41 ml/min, serum creatinine 2.0). In the remaining 10 preeclamptic women serum creatinine clearance in eight of these women was 116 ± 52 ml/min. Therefore in most cases hypocalciuria was out of proportion to the reduction in glomerular filtration rate.

Comment

We originally demonstrated that hypocalciuria is a feature of preeclampsia and may be useful in its diagnosis. In the current study we have extended our investigations of calcium regulation in hypertensive pregnancy. We report for the first time that 1,25-dihydroxyvitamin D levels are significantly lower in preeclamptic women than in normotensive and chronically hypertensive women.

Our finding of lower 1,25-dihydroxyvitamin D levels in preeclampsia and the tendency for parathyroid hormone levels to be higher supports the hypothesis that these alterations in calcium regulatory hormones may be in part responsible for hypocalciuria in this disorder. Indeed, parathyroid hormone–mediated, enhanced distal tubular resorption of calcium is thought to play a role in the hypocalciuria associated with renal insufficiency.[11] More recently, in the rabbit it has been shown that parathyroid hormone causes increased transport of luminal calcium into the cells of the connecting tubule, resulting in reduced urinary excretion of calcium.[12] In this study the increased parathyroid hormone levels observed in many of the preeclamptic women did not result in a statistically significant higher level for the group. However, elevated parathyroid hormone levels in women with preeclampsia have been reported previously by others.[5, 13]

In our earlier study[4] 1,25-dihydroxyvitamin D was measured in 30 of the 40 patients, and although the levels appeared lower in preeclamptic women, the differences between normal, chronic hypertensive, and preeclamptic women were not statistically significant. However, in the earlier study the patients had mild preeclampsia, in contrast to the patients studied in this report who had severe disease. Moreover, in our earlier study patients with transient hypertension and chronic hypertension with superimposed preeclampsia were included in the analysis and may have masked the differences.

In normal pregnancy elevations of 1,25-dihydroxyvitamin D have been consistently observed.[14-17] Although there is still controversy regarding whether parathyroid hormone levels are elevated or decreased during normal pregnancy, more recent studies measuring intact hormone reported lower levels.[18] In this study C-terminal, rather than intact, parathyroid hormone was measured. The concentration of C-terminal parathyroid hormone is determined in part by changes in glomerular filtration rate, whereas intact parathyroid hormone levels best reflect parathyroid gland secretory activity. Because alterations in renal function and alterations in calcium metabolism are present in preeclampsia, it is not yet clear which assay is more relevant to the pathophysiologic characteristics of the disease. We would have preferred to measure intact and C-terminal parathyroid hormone in our patients; however, the intact assay was not available for this study.

The factors responsible for elevated 1,25-dihydroxyvitamin D levels in normal pregnancy are not well understood; therefore at the present time we can only speculate as to the basis for reduced levels in preeclampsia. There is evidence that placental 1α-hydroxylase contributes to the elevated 1,25-dihydroxyvitamin D levels in normal pregnancy.[19, 20] Because preeclampsia, especially when it is severe, is characterized by suboptimal placental function, perhaps as a result of ischemia,[21] the lower 1,25-dihydroxyvitamin D levels may reflect placental dysfunction caused by reduced uteroplacental blood flow. Alternatively, abnormal proximal tubular function, resulting in diminished renal 1α-hydroxylation may be responsible for reduced levels of 1,25-dihydroxyvitamin D.

We have previously reported that nonpregnant individuals with low-renin essential hypertension have elevated 1,25-dihydroxyvitamin D levels.[22] Although normal pregnancy is associated with marked stimulation of the renin-angiotensin system, when preeclampsia develops, plasma renin activity is suppressed.[23] The reason for the differential profile of 1,25-dihydroxyvitamin D in these two conditions may be that in low-

renin essential hypertension the elevation in 1,25-dihydroxyvitamin D is a physiologic response to low ionized calcium and parathyroid hormone–mediated stimulation of renal 1α-hydroxylase, whereas in preeclampsia decreased 1,25-dihydroxyvitamin D is the primary disturbance.

Finally, it is worth mentioning that several institutions are investigating the ability of calcium supplementation to prevent preeclampsia, as a result of reports that dietary calcium deficiency predisposes women to development of preeclampsia.[24] However, if dietary calcium deficiency leads to preeclampsia, then this might be expected to cause elevated parathyroid hormone and stimulation of renal 1,25-dihydroxyvitamin D production. Our observation that 1,25-dihydroxyvitamin D is lower in preeclampsia raises the possibility that decreased intestinal absorption of calcium (as a secondary result of decreased vitamin D) rather than decreased dietary intake may be involved in the pathogenesis of preeclampsia. However, in the setting of reduced 1,25-dihydroxyvitamin D, dietary calcium deficiency might be expected to lead to more profound calcium depletion. Therefore the outcome of calcium supplementation trials (for the prevention of preeclampsia) will be of great interest.

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Long-term sequelae of acute pelvic inflammatory disease

A retrospective cohort study

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OBJECTIVE: Our objective was to prospectively assess the frequency and predictors of long-term sequelae of acute pelvic inflammatory disease.

STUDY DESIGN: We performed a retrospective cohort study of 140 women admitted for treatment of pelvic inflammatory disease to San Francisco General Hospital in 1985, locating and interviewing 51 women by telephone a median of 37 months later.

RESULTS: Twelve (24%) women had pelvic pain for 6 months or more after hospitalization, 22 (43%) had subsequent episode(s) of pelvic inflammatory disease, and 40% were involuntarily infertile. A history of pelvic inflammatory disease prior to admission was associated with infertility ($p = 0.05$), chronic pelvic pain ($p = 0.03$), and pelvic inflammatory disease subsequent to hospitalization ($p = 0.06$). Longer duration of abdominopelvic pain before admission and younger age at the time of first sexual intercourse were associated with both infertility ($p = 0.02$, $p = 0.04$) and subsequent pelvic inflammatory disease ($p = 0.04$, $p = 0.0008$).

CONCLUSIONS: Further studies are needed to more accurately assess the incidence and predictors of long-term sequelae of pelvic inflammatory disease. A reduction in adverse outcome will require more stringent efforts at education and reduction of risk-taking behaviors. (AM J OBSTET GYNECOL 1992;166: 1300-5.)

Key words: pelvic inflammatory disease, infertility, chronic abdominal pain

Approximately 750,000 women in the United States require treatment for acute pelvic inflammatory disease annually.[1] In addition to the morbidity and economic costs associated with acute illness, the risk of long-term sequelae of this infection appears to be substantial. A prospective Swedish study of 415 women with laparoscopically verified acute salpingitis reported frequencies of 21.2% involuntary infertility, 18% chronic abdominal pain, and 4.1% ectopic pregnancy after an episode of acute pelvic inflammatory disease.[2] Seroepidemiologic case-control studies have further implicated acute pelvic inflammatory disease, particularly that caused by *Chlamydia trachomatis*, as a precursor of both infertility[3-5] and ectopic pregnancy.[6-9]

Given a more heterogeneous population and a possible difference in distribution of etiologic pathogens in women in the United States with acute pelvic inflammatory disease as compared with that in the Swedish studies by Westrom,[2] we sought to assess the frequency of long-term sequelae prospectively. We extracted baseline clinical and microbiologic data from review of the medical charts of all women with the discharge diagnosis of acute pelvic inflammatory disease at San Francisco General Hospital in 1985. We then attempted long-term follow-up of all women by telephone interview in 1988 and 1989, to assess the frequency and predictors of long-term sequelae of acute pelvic inflammatory disease.

Methods

All women with the discharge diagnosis of pelvic inflammatory disease, salpingitis, or tuboovarian abscess on the gynecologic, family practice, medical, or pediatric services at San Francisco General Hospital in the year 1985 were identified by computerized retrieval. All women older than 32 at the time of admission were excluded from further analysis, to avoid bias in the assessment of the outcome of infertility.

Data concerning demographic characteristics, birthing and contraceptive histories, clinical presentation at the time of admission, microbiologic pathogens, and complications during follow-up were extracted by review of the medical record. Cervical, endometrial, tubal, or peritoneal specimens were cultured for *Neis-*

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Table I. Baseline characteristics of women with acute pelvic inflammatory disease, San Francisco General Hospital, 1985

	Total (N = 140)	*Interviewed (n = 51)*	*Not interviewed (n = 89)*
Mean age (yr)	23.1 ± 4.3	22.8 ± 4.6	23.3 ± 4.2
Race			
White	31 (22%)	13 (25%)	18 (20%)
Black	78 (56%)	28 (55%)	50 (56%)
Hispanic	20 (14%)	6 (12%)	14 (16%)
Asian	6 (4%)	2 (4%)	4 (4%)
Unknown	6 (4%)	2 (4%)	3 (3%)
Birth control method at time of index infection			
None	96 (69%)	38 (75%)	58 (65%)
Oral contraceptives	8 (6%)	3 (6%)	5 (6%)
Intrauterine device	4 (3%)	1 (2%)	3 (3%)
Diaphragm or foam or sponge	9 (6%)	3 (6%)	6 (7%)
Condoms	4 (3%)	0	4 (4%)
Other or unknown	19 (14%)	6 (12%)	13 (15%)
History of pelvic inflammatory disease	57 (41%)	21 (41%)	36 (40%)
Presence of tuboovarian abscess*	24 (17%)	13 (25%)	11 (12%)
Positive *N. gonorrhoeae* culture	63/127 (50%)	19 (41%)	44 (55%)
Positive *C. trachomatis* culture	32/118 (27%)	14 (32%)	18 (24%)
Mean temperature (° C)	37.8 ± 0.8	37.7 ± 0.7	37.8 ± 0.9
Mean white blood cell count (/mm³)	13.0 ± 5.0	13.8 ± 5.8	12.5 ± 4.3
Mean erythrocyte sedimentation rate	35.3 ± 26.9	38.3 ± 27.2	33.6 ± 26.8
Mean no. of days pain before admission	5.8 ± 7.0	6.9 ± 8.2	5.2 ± 6.1
Mean no. of days in hospital	5.4 ± 1.9	5.7 ± 6	5.2 ± 1.8

Numbers after mean values are standard deviations.
*$p = 0.05$.

seria gonorrhoeae, C. trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, and facultative aerobic and anaerobic organisms, as previously described.[10]

From April 1988 to March 1989 repeated attempts were made to locate all women in the sample. Current addresses were sought through use of the hospital computer, and through formal requests to the Sexually Transmitted Diseases Control Morbidity Registry of the San Francisco Department of Public Health, the Division of Motor Vehicles, and the United States Post Office. We sent initial and follow-up letters to all available addresses, with a self-addressed, stamped postcard enclosed for reply. In addition, all women for whom a telephone number was available were contacted and asked to participate. Attempts to reach the potential participant at each referred phone number were made until met with inability to locate her or refusal to be interviewed; the interviewer's name and phone number were offered so that the participant might be able to call at a time most convenient for her.

The telephone interview was a standardized questionnaire administered by a single individual (S.S.). Long-term sequelae of pelvic inflammatory disease were defined as follows: (1) involuntary infertility, which was failure of conception after a period of at least 1 year of unprotected intercourse (women who were rendered surgically infertile after the index episode of acute pelvic inflammatory disease and those who regularly used contraception to prevent pregnancy were excluded from this component of the analysis), (2) ectopic pregnancy, (3) chronic pelvic pain, which was pain in the pelvic area lasting >6 months and beginning after the index episode of pelvic inflammatory disease, (4) recurrent pelvic inflammatory disease, which was a treated episode of acute pelvic inflammatory disease that occurred after the index episode in 1985. Definitions of involuntary infertility and chronic abdominal pain were the same as those used in previous studies.[2, 11]

Continuous variables are described with mean and standard deviation and analyzed with the two-tailed *t* test or logistic regression. Categoric variables were compared with two-tailed Fisher's exact or χ^2 tests. Relative risk estimates are followed by 95% confidence intervals. All analyses were done with the SAS statistical program (SAS Institute Inc., Cary, N.C.).

The study received approval from the Institutional Review Board of the University of California, San Francisco.

Results

Baseline characteristics. Chart review was performed for each of 140 hospitalized women; baseline characteristics are described in Table I. A history of pelvic inflammatory disease was found in 55 (39%) women; 27 reported one prior episode and 28 had had two or more prior episodes. A history of prior pelvic inflammatory disease, prior gonococcal cervical infection, or prior chlamydial cervical infection was elicited in 77 (55%) women. Fourteen women (10%) admitted

to exchanging in sex for money and 28 (20%) to past or current intravenous drug usage.

Tuboovarian abscess was diagnosed in 16% of women by ultrasonography or physical examination and was associated with a longer mean duration of abdominopelvic pain before the initiation of antimicrobial therapy (8.6 vs 5.2 days, $p = 0.03$). Antimicrobial therapy of acute pelvic inflammatory disease consisted of cefoxitin with doxycyline in 91 women (65%), clindamycin with tobramycin in 31 (22%), and other agents (SCH 34343, ampicillin-sulbactam, mezlocillin) in 18 (13%).

Microbiology. Cervical, endometrial, or tubal specimens yielded *N. gonorrhoeae* in 50% of 127 women and *C. trachomatis* in 27% of 118 women; rate of coinfection was 14% in 113 women tested (Table I). Microbiologic pathogen was not associated with age, method of birth control, presence of cervical mucopus on examination, history of irregular bleeding, or tuboovarian abscess. No differences in temperature or white blood cell count on admission, duration of abdominal pain before initiation of therapy, or number of days of hospitalization were detectable in women with gonococcal infection compared with those having chlamydial infection. Mean erythrocyte sedimentation rate was highest in women with chlamydial infection (52.4 mm/hr) and lowest in women with neither *N. gonorrhoeae* nor *C. trachomatis* isolated (27.4 mm/hr) ($p = 0.0002$, Kruskal-Wallis test).

Endometrial cultures were obtained in 97 women: *Gardnerella vaginalis* was isolated in 50 (52%) and *Bacteroides* species in 30 (31%) (*Bacteroides bivius* 21, *Bacteroides disiens* 6). Peptostreptococci were found in 28 women, peptococci in 17, and mobiluncus in four. Aerobic organisms included lactobacilli (44 women), coagulase-negative staphylococci (24), coryneform bacteria (23), and streptococci (viridans group 14, group D 11, group B 10). *Haemophilus influenzae* was isolated from one specimen. Endometrial cultures were sterile in six women.

Frequency and predictors of long-term sequelae in interviewed women. Fifty-one women (36.5%) were interviewed by telephone at a median interval of 37.5 months after the index episode of pelvic inflammatory disease (range 29 to 49 months). Two women refused to be interviewed and 87 could not be located.

Twenty-five women (48%) had a subsequent pregnancy; of these two (2.4%) had ectopic pregnancies at 17 and 30 months after the index pelvic inflammatory disease episode, respectively.

Seventeen of 42 interviewed women (40%) who were neither surgically infertile nor regularly using contraception met the definition for involuntary infertility. Women with an episode of pelvic inflammatory disease before the 1985 admission were more likely to be infertile (59% vs 27%, $p = 0.05$), as were those with a younger age at the time of first sexual intercourse (mean 14.3 vs 16 years, $p = 0.04$) (Table II). Mean number of prior episodes of pelvic inflammatory disease was 1.3 in infertile women versus 0.6 in others ($p = 0.05$). Women who had ≥2 days of abdominal pain before admission had a twofold increased risk of infertility when compared with women who had pain of <2 days' duration (54% vs 14%, $p = 0.02$). In a multivariate model only a history of pelvic inflammatory disease before the index 1985 episode remained independently associated with involuntary infertility ($p = 0.05$) (Table II).

Twelve of 51 women (24%) had chronic pelvic pain (see Methods). Nine had sought medical attention for the pain; four of these had been treated with antibiotics, two used nonsteroidal antiinflammatory agents, and six took no medication for relief. Four had gynecologic surgical procedures possibly related to their complaints of pain (laparoscopy 2, salpingectomy–salpingostomy–lysis of adhesions 1, percutaneous drainage of ovarian cyst 1). A history of pelvic inflammatory disease before 1985 was associated with occurrence of chronic pain after the index episode ($p = 0.03$) (Table II); no other associations with chronic pain were detectable.

Twenty-two women (43%) had one or more episodes of pelvic inflammatory disease subsequent to the 1985 index episode, occurring at a median of 2.1 months after admission (range 1 to 40). Women with subsequent episodes of pelvic inflammatory disease tended to be younger at the time of first sexual intercourse (mean 13.9 vs 16.4 years, $p = 0.0008$) and had a longer duration of abdominal pain before presentation for admission in the index 1985 episode (mean 8.9 vs 4.3 days, $p = 0.04$). Mean number of pelvic inflammatory disease episodes before 1985 was higher in women with subsequent episodes (1.4 vs 0.4, $p = 0.008$). Risk of subsequent pelvic inflammatory disease was 30% in women with no history of PID, 50% in those with one prior episode, and 72% in those with two or more past episodes. A history of pelvic inflammatory disease before the index 1985 episode ($p = 0.02$), age at the time of first sexual intercourse ($p = 0.01$), and duration of abdominal pain before admission ($p = 0.04$) retained an independent association with recurrence of pelvic inflammatory disease (Table II). The long-term sequelae of infertility and recurrent pelvic inflammatory disease were associated with one another ($p = 0.05$).

Method of contraception, microbial pathogen, presence of tuboovarian abscess, mean temperature, white blood cell count, erythrocyte sedimentation rate, and type of antimicrobial agent administered did not show an association with occurrence of long-term sequelae by univariate analysis in the cohort; however, when stratified for first versus recurrent episode of acute pel-

Table II. Frequency and predictors of long-term sequelae of acute pelvic inflammatory disease

Sequela	*Frequency (No. and %)*	*Risk factor*	*Univariate analysis*		*Multivariate analysis p value*
			p	*Relative risk and 95% confidence interval*	
Involuntary infertility	17/42 (40%)	History of pelvic inflammatory disease	0.05	1.8 (1.0-3.3)	0.05
		Age at time of first sex	0.04		0.39
		≥2 Days of pain before theapy	0.02	2.0 (1.1-3.6)	0.07
Chronic pelvic pain	12/51 (24%)	History of pelvic inflammatory disease	0.03	1.5 (1.0-2.2)	
Pelvic inflammatory disease after index episode	22/51 (43%)	History of pelvic inflammatory disease	0.06	1.7 (0.9-3.1)	0.02
		Mean No. of days of pain before therapy	0.04	—	0.04
		Age at time of first sex	0.0008	—	0.01
Ectopic pregnancy	2/51 (2.4%)	*			

*Risk analysis not performed because of small numbers involved.

vic inflammatory disease, presence of a positive culture for *C. trachomatis* was significantly associated with involuntary infertility in women in whom the index episode was the first ($p = 0.008$, relative risk = 2.5, 95% confidence interval = 1.0 to 6.2) but not in women who were having a recurrent episode of pelvic inflammatory disease at the time of admission in 1985 ($p = 0.5$). Also, presence of coinfection with *N. gonorrhoeae* and *C. trachomatis* in women with first-episode pelvic inflammatory disease in 1985 was associated with subsequent occurrence of episodes ($p = 0.007$, relative risk = 1.4, 95% confidence interval = 1.0 to 2.3) but not in women with a history of pelvic inflammatory disease at the time of admission in 1985 ($p = 0.4$).

Long-term sequelae in women not interviewed. Comparison of the 51 women who were interviewed by telephone with the remaining 89 women in the cohort revealed no significant differences in baseline characteristics, except that a greater proportion of interviewed women had a tuboovarian abscess during the index admission (25.5% vs 12.4%, $p = 0.05$) (Table I). However, tuboovarian abscess was not associated with occurrence of long-term sequelae in this analysis (infertility $p = 0.5$, chronic pain $p = 0.7$, recurrent pelvic inflammatory disease $p = 0.7$).

Review of the medical record revealed that six of the 89 women not interviewed had sought medical evaluation for inability to conceive. Nineteen had pregnancies subsequent to the index diagnosis of acute pelvic inflammatory disease; one of them was an ectopic pregnancy occurring 13 months later. Ten women were evaluated for complaints of chronic pelvic pain ≥6 months after the index episode, and 19 had recurrent episodes of acute pelvic inflammatory disease after the index admission.

Frequency estimates of long-term sequelae derived solely from chart review data were substantially lower than those derived from telephone interview: infertility 5% vs 40% ($p = 0.0001$), ectopic pregnancy 0.7% vs 2.4% ($p = 0.24$), chronic pelvic pain 13% vs 24% ($p = 0.05$), recurrent pelvic inflammatory disease 27% vs 43% ($p = 0.01$).

Comment

We assessed the frequency of long-term sequelae over a period of 1825 woman months after hospitalization for acute pelvic inflammatory disease in 1985 (median interval after hospitalization 37.5 months). Interpretation of results is limited by an incomplete rate of follow-up in the cohort, failure to confirm the clinical diagnosis of pelvic inflammatory disease laparoscopically, and lack of a control group. Nevertheless, the high frequency of long-term sequelae in this population of women (infertility 41%, chronic pain 43%, recurrent episodes 24%, and ectopic pregnancy 2.4%) in spite of hospitalization for close observation and parenteral antimicrobial therapy suggests that further research is necessary to document the actual incidence of these sequelae and to further our understanding in methods of prevention.

We demonstrated the absence of significant differences in baseline characteristics in women who were interviewed as compared with those not able to be located (Table I), other than the presence of tuboovarian abscess at the time of admission in a greater proportion of women not interviewed. Use of record review alone, however, in women not available for interview yielded markedly lower estimates of long-term sequelae than in those interviewed (infertility 5%, chronic pelvic pain 13%, recurrent pelvic inflammatory disease 27%, ectopic pregnancy 0.7%). These differences may reflect an underestimation of long-term sequelae by record review because of the failure of women in this population to seek evaluation for symptoms or their use of alternative medical centers. Alternatively, overestimation of the frequency of long-term sequelae may have occurred in interviewed women as a result of reporting bias. Therefore our estimates may be construed as minimum (in the noninterviewed group) and maximum (in those interviewed) estimates of long-term sequelae, to be more precisely delineated by careful prospective studies.

We found that a history of one or more episodes of pelvic inflammatory disease before the index admission, present in 41% of patients at the time of admission, was associated with higher rates of involuntary infertility, chronic pelvic pain, and recurrent episodes of pelvic inflammatory disease after the 1985 admission. This is in agreement with previous studies.[2, 12] In spite of interaction with the health care system in 55% of interviewed women for prior pelvic inflammatory disease or gonococcal or chlamydial cervical infection, more than two thirds of women were not using contraception at the time of admission and a maximum of 9% were using barrier contraception. Factors other than prior pelvic inflammatory disease that were associated with an increased frequency of long-term sequelae, such as a younger age at the time of first sexual intercourse and a longer duration of pelvic pain before presentation for medical evaluation, suggest that further progress in the prevention of sequelae must be accomplished through educational efforts directed at reduction of risk-taking behaviors and early recognition of symptoms by the patient.

We were unable in this study to definitively establish the temporal sequence of infertility and acute pelvic inflammatory disease or to establish the cause of involuntary infertility as tubal. In addition, some authors have suggested that the definition of infertility used in this and previous[2, 11, 12] studies may overinflate estimates of frequency.[4] Nevertheless, the correlation of number of episodes of pelvic inflammatory disease with infertility helps to validate the strong link between acute pelvic inflammatory disease and involuntary infertility identified in previous prospective studies.[2, 11, 15-18] Also, the greater frequency of infertility in women with ≥2 days of abdominal pain before initiation of therapy agrees with previous animal[19] and human[20] studies that emphasize the importance of early therapy in the prevention of irreversible tubal damage, a condition that occurs in approximately 2 million women in the United States.[21]

The occurrence of ectopic pregnancy in two interviewed women after the index episode of pelvic inflammatory disease constitutes an increased frequency eightfold over that observed in women of reproductive age in the general United States population.[22] This, combined with evidence from several cohort[2, 23] and case-control[6, 8, 9] studies suggesting a link between salpingitis and subsequent ectopic pregnancies, is evidence that intensification of efforts to prevent pelvic inflammatory disease will help to prevent a condition associated with substantial mortality in this country.[22]

Previous studies have suggested that nongonococcal salpingitis, particularly that caused by *C. trachomatis*, may incur a greater impairment in subsequent fertility than other microbiologic pathogens produce.[2-5, 15, 24] Although we were unable to detect an association of microbiologic pathogen with reproductive outcome in the total group of women studied, we did find an association of chlamydial infection with subsequent infertility in those women in whom the 1985 episode of pelvic inflammatory disease was reported to be the first. It is conceivable therefore that this relationship was masked in the entire interviewed group as a result of confounding by recurrent episodes of tubal damage caused by infection or other strong risk factors. Unfortunately, it does not appear from this or prior studies[25] that alterations in antibiotic therapy to empirically treat chlamydial infection will have a major impact on fertility prognosis.

Future studies should seek to confirm our frequency estimates and to better identify predictors of the long-term sequelae of acute pelvic inflammatory disease. Ideally, laparoscopic or endometrial biopsy confirmation of the clinical diagnosis of acute pelvic inflammatory disease, thorough sampling of involved sites to determine microbiology, and aggressive attempts at optimal follow-up over a long-term period should occur. Consent for enrollment into long-term cohort studies must be obtained at the outset, and intensive educational counseling as to recognized risk factors for recurrence and the importance of initiation of early therapy for symptoms of recurrent pelvic inflammatory disease should be instituted. In addition, exclusion of women who are involuntarily infertile at the time of admission for acute pelvic inflammatory disease is necessary. Extensive investigation of the etiology of infertility identified during follow-up, in both patients (e.g., by assessment of tubal morphologic characteristics by

hysterosalpingogram or laparoscopic dye insufflation) and their partners, should be performed to more accurately define the incidence and natural history of the reproductive sequelae of acute pelvic inflammatory disease.

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LETTERS TO THE EDITORS

Infarction of placental chorioangioma and associated regression of hydrops fetalis

To the Editors: I read the article by Chazotte et al. (Chazotte C, Girz B, Koenigsberg M, Cohen WR. Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis. AM J OBSTET GYNECOL 1990;163:1180-1) with great interest. The authors reported a case of spontaneous regression of a chorioangioma associated with partial resolution of fetal hydrops with a good neonatal outcome.

The authors did not provide any positive evidence that the regression of the hydrops resulted from the infarcted chorioangioma rather than from the healing of the perforated intestine (meconium peritonitis). Regarding this association, my suggestion is that if fetal echocardiography had been performed it might have strongly supported the viewpoint of this article by showing hemodynamic changes.

Because the progression from a normal status to hydropic change is caused by a chorioangioma that acts as a high-volume arteriovenous shunt to produce a volume overload and cardiac failure in the fetus,[1, 2] any improvement in cardiac decompensation will result in a resolution of the hydrops. We have experienced one case of chorioangioma (7 × 7 × 7.8 cm). A fetal echocardiographic examination was performed once a week beginning with the twenty-fourth gestational week, when the chorioangioma was initially diagnosed. The hydrops developed at the thirty-second gestational week, when the aortic and pulmonary blood flow was noted to have increased to a value two times above normal (720 vs 316 ml/min in the aortic flow, 900 vs 422 ml/min in the pulmonary flow). The hemodynamic changes from an intracardiac Doppler study could have proved whether the placental chorioangioma had a major role in the development of the hydrops fetalis and vice versa, whether it played a role in the regression of the hydrops fetalis.

Chazotte et al. also stated that gradual regression of the placental tumor was seen in the serial ultrasonographic examinations. According to the description in their article, the size of the chorioangioma was measured initially at 5 × 6.5 × 5.5 cm and decreased to 5 × 5 cm. This does not seem to be a clinically significant change.

Finally, I am concerned about the "focal" infarction of chorioangioma. In those cases of placental chorioangioma at our hospital, focal infarction is commonly seen in pathologic specimens. I also wonder if the focal infarction could have had a major effect on the cardiovascular changes in fetal circulation and on the subsequent regression of the hydrops fetalis.

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Reply

To the Editors: We appreciate Hsieh and Soong's interest in our case report. We agree that we did not provide positive evidence that the regression of the hydrops fetalis resulted from the infarction of the chorioangioma rather than from healing of the perforated intestine. However, the findings of polyhydramnios and skin edema are unusual in meconium peritonitis in the absence of bowel obstruction[1, 2]; therefore, the hydrops fetalis was more likely to have been the result of the placental tumor.

As illustrated by the case described by Hsieh and Soong, the use of intracardiac Doppler flow studies might have been helpful in determining the cause and the regression of the hydrops in our case. We did not perform such studies with our patient.

With regard to the size of the tumor, the initial dimensions on ultrasonographic examination were 5 × 6.5 × 5.5 cm. Serial sonography revealed that the tumor became smaller, less well visualized, and finally, was recognizable only as a thickened area of placenta without distinct vasculature. In fact, the lesion on the delivered placenta, although measuring 5 × 5 cm, was flattened and infarcted. Thus the volume, nature, and composition changed significantly during the pregnancy. We speculate, as suggested, that the focal infarction of this tumor did play a major role in the resolution of the hydrops fetalis.

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More on early detection of preeclampsia

To the Editors: A critical review of available methods for the early detection of preeclampsia was recently published by O'Brien.[1] That Dekker and Sibai (Dekker GA, Sibai BM. Early detection of preeclampsia. AM J OBSTET GYNECOL 1991;165:160-72) view the topic as im-

portant enough to warrant a repetition so soon may well be a testament to the ongoing importance of the topic.

I am unaware of Dekker's personal biases, but it is genuinely reassuring to read that Sibai is steadfast in his vehement opposition to the potential clinical utility of the second-trimester mean arterial pressure (MAP).

Thus it was with a good deal of amusement that I noted that any reference to our work concerning the clinical utility of the second-trimester MAP had been assiduously omitted. In 1988 we presented new data from a prospective study validating second-trimester MAP as an accurate and effective predictor. These results were presented at the national meeting of the American Federation for Clinical Research and the Sixth International Congress of the International Society for the Study of Hypertension in Pregnancy held in Montreal, Canada, in 1988 and were published in 1989[2] and 1990.[4]

No information about the dates of submission and revision for Dekker and Sibai's paper were provided, but Sibai certainly was aware of our work; he attended our presentation at the Montreal meetings in 1988 and roundly criticized our work there. Dekker and Sibai may not agree with our findings. They may be critical of our methods. They may even have missed our paper in the June 1989 issue of *Obstetrics and Gynecology*. Whatever the case, by omitting reference to our work they have failed to provide the readers of the JOURNAL with all the "Current Developments" for the early detection of preeclampsia.

We screened 730 pregnant women at or near 20 weeks of amenorrhea, calculating the screening second-trimester MAP from Korotkoff phases I and V measured in the seated position. Because we had previously documented that adverse outcomes attributable to maternal hypertension were restricted to women with antepartum hypertension,[4] we used antepartum hypertension as the outcome for our study. This represents a more stringent outcome than pregnancy-induced hypertension or preeclampsia because it includes only these cases documented before labor or delivery[4]; those cases account for 45% of all cases of hypertension-complicating pregnancy.[5] The screening second-trimester MAP level of 85 mm Hg proved to be the optimal cutoff for second-trimester MAP as a screening test with sensitivity of 88%, specificity of 84%, positive predictive value of 21.7%, and negative predictive value of 99.3%. Controlling for the value of the screening second-trimester MAP, the only other important predictors of antepartum hypertension were chronic hypertension and diabetes mellitus. With these three variables, the probability that an individual woman will develop antepartum hypertension can be assessed with a high degree of accuracy (84.5%) by 20 weeks of amenorrhea. When women with either chronic hypertension or diabetes mellitus were excluded from the multivariate analysis, second-trimester MAP remained the major predictor of antepartum hypertension ($p < 0.0001$), with the primigravid state and black race being of comparatively minor importance ($p < 0.01$ and $p < 0.05$, respectively).

Assessment of risk for antepartum hypertension with the screening second-trimester MAP is noninvasive, easily transportable, simple, and inexpensive. By combining the screening second-trimester MAP with historic information about chronic hypertension and diabetes mellitus, three distinct levels of risk have been defined:

The low-risk group includes women with screening second-trimester MAP <85 mm Hg with or without diabetes mellitus or chronic hypertension. Antepartum hypertension developed in 0.7% of these women, and antepartum hypertension in combination with maternal and/or infant morbidity occurred in 0.2% of them.

The moderate risk group includes women with second-trimester MAP ≥85 mm Hg in the absence of diabetes mellitus or chronic hypertension. Antepartum hypertension developed in 13.8% of these women, and antepartum hypertension in combination with maternal and/or infant morbidity occurred in 6%.

The high-risk group includes women with second-trimester MAP ≥85 mm Hg in combination with chronic hypertension or diabetes mellitus. Antepartum hypertension developed in 82% of these women, and antepartum hypertension in combination with maternal and/or infant morbidity occurred in 58.8% of them.

This prognostic stratification provides a simple clinical tool for focusing clinical and research resources on subsets of moderate and high-risk women who would be the most likely to benefit.

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Reply

To the Editors: It is unfortunate to read that Ales is under the impression that I have certain personal biases either against her work or against her endless obsession with second-trimester MAP. In fact, I found her comments to be neither amusing nor accurate.

The title of our review was "Early detection of preeclampsia," with the emphasis on the word "preeclamp-

sia." Unfortunately, Ales is not an obstetrician, and thus she continues to equate the word "hypertension" with preeclampsia. This was true about 10 years ago; however, recent evidence indicates that there is more to preeclampsia than hypertension.

Ales' cited work involves using one reading at ≤20 weeks' gestation as a screening for second-trimester MAP.[1] In contrast, most data in the literature use the average readings of several values obtained during the second trimester. Her data would have been included if the title of our review was "Early Detection of Hypertensive Disorders of Pregnancy" rather than preeclampsia. Indeed, her prospective study was originally included but had to be deleted because it does not apply to preeclampsia. Fortunately, the original critique of her study was still in the original long manuscript and will thus be set forth here:

A recent prospective study by Ales et al.[1] evaluated the predictive value of one MAP recording at 20 weeks' gestation in 730 women. The authors found a MAP value of ≥85 mm Hg to be clinically significant in predicting antepartum hypertension. Of the 139 women with a MAP ≥85 mm Hg, 21.6% had subsequent antepartum hypertension as compared with a frequency of 0.7% among the women who had a value of <85 mm Hg. Although these results are impressive and the authors used sophisticated multiple logistic regression analysis, the clinical value of this study is doubtful because it only demonstrates that an elevated MAP can be found in the majority of pregnant women in the second trimester along with other well-known risk factors for antepartum hypertension. Among the 139 women with a second-trimester MAP ≥85 mm Hg, the incidence of previous history of hypertension was 39%, a history of chronic hypertension was 12.9%, a history of diabetes mellitus of 2.9%, and a history of perinatal mortality of 15.6%, versus respective rates of 8.5%, 1.2%, 1.0%, and 7.3% in the group with MAP <85 mm Hg. In addition, the study included only six patients with actual preeclampsia (proteinuric hypertension, according to the authors' definition). Thus the conclusions of this study support those of Chesley and Sibai,[2] where it is stated that increased second-trimester MAP predicts transient antepartum hypertension rather than preeclampsia.

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Details of electronic fetal monitoring randomized control trials

To the Editors: The randomized control trials on the efficacy of electronic fetal monitoring have widely been interpreted as showing that auscultation and electronic fetal monitoring are "equivalent."[1] Detailed analysis of electronic fetal monitoring patterns from several of these studies, however, tend to betray that interpretation.[2-5]

In both the article by Larson[5] from the premature randomized control trials and the article by Ellison et al. (Ellison PH, Foster M, Sheridan-Pereira M, MacDonald D. Electronic fetal heart monitoring, auscultation, and neonatal outcome. AM J OBSTET GYNECOL 1991;164:1281) with data from the Dublin trial, the authors find an association between adverse outcome and abnormal fetal heart rate (FHR) patterns, but no such correlation with auscultation. This suggests that we cannot rely on even assiduous auscultation for insight into the obstetric (fetal) contribution to neonatal depression and injury.

Ellison et al. emphasize the importance of synthesizing the various components of neonatal evaluation into an estimate of severity and prognosis. Their descriptive classification of FHR patterns characterizes more than 50% as abnormal and more than 20% as ominous; this classification system precludes any understanding of the mechanism, severity, or duration of any fetal insult. The authors imply that the pattern interpretation influenced the operative delivery rate, but they provide few insights. Were ominous patterns present from the outset of labor, were they acted on, and how long was the interval before intervention? The article inappropriately invites the inference that all the adverse outcomes associated with abnormal heart rate patterns developed acutely during labor, not before.

In their introduction the authors suggest that FHR patterns may distinguish the fetus with chronic injuries, but their research attempts no such separation. With the access to FHR tracings, clinical history, and follow-up data, the authors have the unique opportunity to attempt to distinguish those fetuses whose adverse outcome likely preceded labor from those whose handicap likely resulted from asphyxial or mechanical complications of labor and delivery. In others, analysis might reveal no obvious relationship between obstetric factors and outcome. The authors might consider the following scheme: Late, variable, or prolonged decelerations associated with decreased or absent variability from the onset might represent an ongoing asphyxial condition, the interruption of which may not change outcome. Such decelerations developing in a fetus with a previously normal pattern suggest an acute asphyxial event *potentially* amenable to correction or rescue. Persistently absent variability not associated with significant decelerations suggests preexisting abnormality (injury, anomaly) without current hypoxia. Intervention here is unlikely to change the outcome.[6-10]

These subsidiary reports from the randomized control trials clearly imply a benefit to electronic fetal monitoring, at least for the prediction of outcome.[8-10] Only the availability of predictive data on the individual fetus will enable us to understand both the mechanism and timing of insult and the potential for remediation, if any. Each of the randomized control trials raises the

issue of the adequacy of bedside interruption of the tracings. Neither randomized control trial discussed here supports the notion that auscultation and electronic fetal monitoring are equivalent.

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Reply

To the Editors: Schifrin indicates that our work showed no correlation between adverse outcome and auscultation. That is not so. In Table VI, the association is shown between FHR abnormalities as assessed by auscultation and the neurologic examinations (an excellent measure of adverse outcome) at 0 to 48 hours and 72 hours to 1 week. For the subsample monitored by auscultation who received neurologic examinations, *FHR abnormality* was the leading and statistically significant indicator of neurologic abnormality at both the 0 to 48 hour ($t = 0.01$) and the 72 hour to 1 week examinations ($t = 0.05$). Many other labor and delivery variables were entered into these data analyses. For the subsample monitored by electronic fetal heart monitoring that received neurologic examinations, late decelerations were the leading indicator of neurologic abnormality at both 0 to 48 hours ($t = 0.00$) and 72 hours to 1 week ($t = 0.00$). Marked bradycardia also was significantly associated with the neurologic evaluation at 72 hours to 1 week ($t = 0.00$).

To analyze the data in the additional ways suggested by Schifrin, the original fetal heart monitoring strips from the Dublin study would need to be reinterpreted in a different manner. As we suggested in the article, trend analysis would probably be more useful for this purpose. Unfortunately, our research team in Denver has not had access to the original tapes, nor have we funding for such a study, however exciting it might be.

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Enhanced sensitization after cordocentesis

To the Editors: Recently MacGregor et al. (MacGregor SN, Silver RK, Sholl JS. Enhanced sensitization after cordocentesis in a rhesus-isoimmunized pregnancy. AM J OBSTET GYNECOL 1991;165:382-3) reported a case in which cordocentesis was associated with a marked increase in the maternal indirect Coombs' titer and the subsequent development of immune hydrops. MacGregor et al. suggest that the risk of cordocentesis increasing the maternal sensitization is greater than that of amniocentesis. They made several assumptions or errors in arriving at their conclusion. First, the maternal antibody titer is a reasonable indicator of the fetal risk only during the first sensitized pregnancy.[1] In subsequent pregnancies the variability is too great. Second, at 25 weeks and 3 days they obtained a fetal hematocrit that was already below the 2.5th percentile for gestational age. In other words, the fetus already had anemia. On the basis of our experience, currently consisting of 150 alloimmunized pregnancies, such a fetus has a 90% chance of requiring transfusion therapy if the action line for transfusion is set at 30%.[2,3] The likelihood of the severe anemia developing is so great that these mothers are seen weekly and the fetal blood sampling repeated initially in 7 to 10 days. That this fetus developed a profound anemia is not surprising. An elevated fetal reticulocyte count and total bilirubin level would further increase the index of suspicion. Third, although there is a relationship between the fetal direct Coombs' titer measured initially and hemolytic anemia, it is by no means strong.[2,3] The change in the fetal direct Coombs' titer with advancing gestation has not been studied. Fourth, the authors provide no evidence that cordocentesis is associated with a greater risk of enhanced sensitization than amniocentesis. Abrupt increases in the degree of sensitization after amniocentesis are well documented. We have longitudinally examined approximately 40 pregnancies in which the initial maternal titers were <128 at the time of the first cordocentesis. In only two instances has a ≥ 2-tube dilution increase in the antibody titer been detected during the course of the pregnancy. In both of those instances, the fetal hematologic status was unchanged. We have shown that when a needle guide is used to prevent lateral movement of the needle, the change in maternal serum α-fetoprotein after cordocentesis is similar to that reported for amniocentesis.[4] This differs from the results obtained with a free-hand technique where the increases are larger.[5] As with amniocentesis, it is usually possible to avoid the placenta by performing

the cordocentesis at a midloop. When that is done the risk of worsening sensitization from cordocentesis appears to be no different from that associated with amniocentesis.

In summary, the patient assessed by MacGregor et al. fell into a high-risk category; her worsening fetal hemolytic anemia was predictable from the outset. Although sensitization might have been exacerbated by placental puncture, it is the way the procedure was performed in this instance rather than the procedure itself that is of concern.

Carl P. Weiner, MD

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Reply

To the Editors: We thank Weiner for his interest in our article. We agree that a low maternal antibody titer cannot be used to ensure the absence of fetal risk after a previously affected pregnancy, and we therefore offered the patient either amniocentesis or cordocentesis. However, the report that was the basis for this management does not imply that a higher titer poses no greater risk for the fetus.[1] We expect that a maternal titer of 1:4096 (after initial cordocentesis) is more likely to be associated with severe fetal anemia than a titer of 1:8 (before cordocentesis). At initial sampling the fetal reticulocyte count was 15.4%, direct Coombs titer 1+, and hematocrit 30.9%. Our experience also suggests that such a fetus is likely to have the hematocrit decrease to <30% and require transfusion. However, in such cases monitored with weekly ultrasonographic examinations and repeat cordocentesis, we have not seen an additional fetus develop hydrops and decrease the hematocrit to 12% in a 3-week period. A decline in the hematocrit to <30% is much different from 12%. Data are not available from the report by Weiner et al. addressing the rapidity of hematocrit decline in patients such as we described, although we suspect that our case is uncommon.[2] No data are available comparing the risk of enhanced sensitization after amniocentesis versus cordocentesis. Although Weiner et al. demonstrated little increase in maternal serum α-fetoprotein levels after cordocentesis, avoiding transplacental needle entry,[3] we are unaware of the correlation between elevation of maternal serum α-fetoprotein and the risk of enhanced sensitization in alloimmunized pregnancy. A secondary immune response may occur after introduction of <1 ml of antigen-positive red blood cells. Maternal serum α-fetoprotein elevation would likely occur only with much greater fetal blood contamination. This study did not compare needle-guided cordocentesis with either amniocentesis or free-hand cordocentesis; therefore, it is not possible to assess the relative risks of fetal blood entering the maternal circulation. Finally, we agree that placental puncture during cordocentesis may be associated with risks and avoided if feasible; however, needle placement in a free loop of umbilical cord may also be associated with risks, including technical difficulty of cord movement and more frequent dislodgment, particularly if transfusion is necessary.

Although cordocentesis has improved both evaluation and therapy of fetuses with red blood cell alloimmunization, potential risks such as enhanced sensitization must be considered. As other reports have suggested, midtrimester amniocentesis should continue to be an option in the evaluation of these fetuses.[4]

Scott N. MacGregor, DO, Richard K. Silver, MD, and John S. Sholl, MD

Section of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Evanston Hospital, Evanston, IL 60201

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Increasing use of gynecologic ultrasonography

To the Editors: I read with great interest the ultrasonography panel developed by the American Gynecological and Obstetrical Society, especially the article by Willson (Willson JR. Ultrasonography in the diagnosis of gynecologic disorders. AM J OBSTET GYNECOL 1991;164:1064-71.) in which my work was discussed. Willson's material was well thought out and timely, and it gave me some new perspectives. Still I feel obligated to comment.

First, one constant theme has to do with the "cost-effectiveness" of increasing use of gynecologic ultra-

sonography with the vaginal probe. All of the analyses and comments seem to assume that every time the probe is used the patient incurs a separate, additional charge of $150 to $200. This need not be true. The ultrasonographically enhanced bimanual examination adds very little time, and as vaginal probe equipment becomes smaller and dedicated and is used more routinely the *true* costs will be a mere fraction of what people currently charge.[1] This will be especially true in terms of our concerns about earlier diagnosis of ovarian cancer. We already screen for this disease: it is called the annual gynecologic visit. Admittedly this has an inadequate pick-up rate in the early stages of the disease. However, as vaginal probe–enhanced bimanual examination becomes more widespread, these will be an improvement in detection. Once again, the dollar numbers quoted are not true indicators of cost, either current or future.

Second, Willson correctly points out that most reports on the use of ultrasonography in gynecology come from those like myself who have some background in diagnostic imaging. Currently many practitioners are learning imaging at postgraduate courses and then going out and creating their own learning curves. Thus Willson believes the diagnoses made by this group are less accurate than those made by the imaging professionals. Initially this may be true. However, the beauty of the vaginal probe is that it *is* a diagnostic modality. A new entrant into the field can and should have a variable learning curve. Although physicians acquire experience and confidence with their own diagnoses generated with vaginal probe ultrasonography, they can and should continue to triage such patients as they did before they used the probe. This is not an all-or-none phenomenon. We continue to improve our wealth of knowledge, experience, and skill over our entire professional lives. Furthermore, the real key to upgrading physicians skills in this country lies in residency training. Every resident who finishes a program without the necessary skills becomes part of the problem, not the solution. It will take an entire generation of obstetricians and gynecologists to fully implement such new technology, but certainly in our institution it is well on its way.

Finally, Willson makes the point that identification of small myomas, ovarian enlargements, and physiologic cysts will lead to increased patient concern and unnecessary operations. I share this concern greatly. The biggest potential problem with this new technology is overinterpretation of such findings and merely applying old clinical approaches to information obtained with such increasingly refined technology. Along with the introduction of such technology, education of both physicians and patients is mandatory. I realized this very early on and have committed myself to it. For instance, use of words like "cyst" need to be rethought. It is petrifying to the uneducated patient. It merely means "filled with fluid," is easily seen on ultrasonography and says nothing about cause or significance. I appreciate Willson's help in this arena, but the answer is not to abandon the technology, but rather to do further appropriate studies to responsibly incorporate the technology into routine patient care.

Steven R. Goldstein, MD
New York University Medical Center, 530 First Ave., Suite 5E, New York, NY 10016

REFERENCE

1. Goldstein SR. Incorporating endovaginal ultrasonography into the overall gynecologic examination. AM J OBSTET GYNECOL 1990;162:625-632.

Reply

To the Editors: I appreciate Goldstein's comments. He, of course, is one of the leaders in the use of diagnostic ultrasonography by obstetrician-gynecologists.

Goldstein is less concerned than I and others over the cost of ultrasonography and other examinations when they add little to diagnostic accuracy. Ultrasonographic equipment must be paid for; as technology improves new machines must be purchased and paid for, not by the physicians who use them, but by their patients. I doubt that the additional charge for office visits that includes ultrasonographic examinations will be eliminated after the equipment is amortized. Hillman et al. calculated that physicians who own ultrasonographic equipment use diagnostic imaging 4.0 to 4.5 times more often than those who refer patients to radiologists. In addition, the charge for obstetric ultrasonographic examinations by self-referring physicians averages $178 per examination as compared with $25 by radiologists. Hillman et al. state, "The differences between self-referring physicians and radiologist-referring physicians in the use of imaging are so great that some concern over the role of financial incentives must be invoked."

I agree with Goldstein and stated in my article that the accuracy of ultrasonographic diagnosis will improve with advancing technology and increased experience in interpreting the images.

Goldstein seems to agree with my statement that "When expertise in this important adjunctive examination is expanded and when it is taught in departments of obstetrics-gynecology by obstetrician-gynecologists, most residents will become as competent in gynecologic ultrasonographic diagnosis as they now are in obstetrics."

The last sentence of Goldstein's letter seems to imply that I suggested that ultrasonography be abandoned. This does not in any way reflect what I believe or said, which is simply that at the moment it appears that ultrasonography is being overused and that its best use is as a secondary, not a primary, diagnostic tool.

J. Robert Willson, MD
Department of Obstetrics and Gynecology, University of New Mexico Hospital, 3211 Lomas Blvd. NE, Albuquerque, NM 87131

REFERENCE

1. Hillman BJ, Joseph CA, Mabry MR, et al. Frequency and cost of diagnostic imaging in office practice—a comparison of self-referring and radiologist-referring physicians. N Engl J Med 1990;393:1604-8.

At last: An objective observation

To the Editors: Congratulations to Dr. Ward on his article (Ward CJ. Analysis of 500 obstetric and gynecologic malpractice claims: causes and prevention. AM J OBSTET GYNECOL 1991;165:298-306). Although I am not sure exactly how Dr. Ward identified the cases he defined as "defensible" or "undefensible," the most important aspect of the article to me was that physicians are now able to admit that—no matter what the percentages are—negligence, malpractice, deviation from the standard of care, and bad doctors, call it what you will, do occur.

Dr. Ward has expended great energy looking at cases and trying to make sense of them; for that I congratulate him. It is refreshing to read a more objective response to the malpractice issues than the usual party-line drivel that is given to physician readers.

On the other hand, Dr. Freedman's inflammatory comment decrying the fact that the crisis becomes "so terribly lucrative to one other profession," is the response that ignores the medical profession's problem and projects derogation toward the legal profession.

Finally, Dr. Blanchard's insinuation (promulgated by others) regarding any and all medical experts in malpractice trials (somewhat derogatorily referred to as "these people"), that good, upright, proper, outstanding physicians would never dare speak out critically of fellow physicians, is also typical of the profession's attitude and should be strongly denounced. Her anecdotal and apocryphal comments that, ". . . we have medical experts stating that the patient had an enema and that this caused the baby to be brain damaged . . ." is pure hockey puck.

Please keep up the good work in providing information about the medical negligence area to your readers.

William J. Morton, MD, JD

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Response declined

Examination of placentas by pathologists

To the Editors: Goldman (Goldman H. Examination of placentas by pathologists (Letter). AM J OBSTET GYNECOL 1991;165:783) and Salafia and Vintzileos (Salafia CM, Vintzileos AM. Why all placentas should be examined by a pathologist in 1990. AM J OBSTET GYNECOL 1990;163:1282-93) have widely differing opinions relative to indications for placental examination. The College of American Pathologists held a conference on the examination of the placenta and published a report. Your readers may be interested in the therein recommended "indications for placental examination."[1]

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REFERENCE

1. Altshuler G, Deppisch L. College of American Pathologists Conference XIX on the examination of the placenta: report of the working group on indications for placental examination. Arch Pathol Lab Med 1991;115:701-3.

Animals and ethics: How society's attitudes have changed

To the Editors: There has been a drastic change in the social and philosophic Zeitgeist and with it our attitudes toward the treatment of animals. Criticism of the use of animals in biomedical research no longer can be brushed aside merely as antiscientific or antiintellectual. J.M. Maharry correctly observed, in his presidential address, "The issue of animal rights and human rights" (AM J OBSTET GYNECOL 1991;164(6):1543-8), that researchers and members of the medical profession can no longer afford merely talking to each other about the fact that "60 million Americans do not approve the use of living animals in research." What's urgently needed is careful listening to what other segments of society think about this highly controversial issue, rather than embarking on a high-tech advertising campaign in support of the status quo, as Maharry proposes.

That animals, in fact, have interests, because they, like humans, can suffer, and thus have an interest in avoiding suffering, is a subject being argued by students of philosophy throughout the United States and elsewhere. An increasing number of articulate philosophers have demonstrated in their writing, "a coming maturity in animal ethics that can't be dismissed as either sentimentalist or scientifically uninformed," as stated by Burghardt.[1] We are less and less on firm philosophic ground if we continue to adhere to the anthropocentric view that all nonhuman life exists only for man's use and benefit. The paucity of contemporary philosophy in firm support of this anthropocentric belief is striking. Ethology, neurobiology, and other disciplines have taught us that animals share with us many cognitive and perceptual attributes that make highly invasive procedures no longer merely an issue of freedom to conduct research. Even the issue of potential human benefit would appear in a different light under these circumstances.

Maharry reiterated the self-serving claims by animal researchers that without animals there would not be "a single modern and substantial item of medical knowledge." Consider the cure of childhood leukemia and lithium for the treatment of manic-depressive illness, two examples frequently attributed to animal research.[2] Yet medical historian Brandon Reines has discovered that patient-centered clinical investigation was behind these success stories.[3]

Finally, Maharry is simply misinformed for failing to see that the animal rights movement encompasses concern for the environment and a host of other human rights issues. A recent survey conducted at Oregon

State University under a grant by the Putting People First Foundation[4] confirmed just that.

Ulrich Fritzsche, MD
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2. Horner C. Animal rights and wrongs. Newsweek 1990 Jan 22:12.
3. Reines B. On the locus of medical discovery. J Med Philos 1991;16:183-209.
4. Public affairs. Physiologist 1990;33:183.

Reply

To the Editors: As is so typical of zealots, they defend their position by attacking the motives of their perceived enemies. I refer to Fritzsche's statement, "self-serving claims by animal researchers." To validate this statement, he implies that childhood leukemia and manic-depressive illness were cured without the benefit of animal research. His inference that no animal studies were involved in effecting the cure of these two diseases is either hopelessly naive or it is deliberate misinformation.

Consider the drugs used in the treatment of the two diseases: lithium carbonate in the treatment of manic-depressive illness and various combinations of doxorubicin, vincristine, prednisone, asparaginase, 6-mercaptopurine, and methotrexate in the treatment of childhood leukemia. It is ludicrous to suggest the Food and Drug Administration would release these drugs for clinical study had there not been prior, and extensive, testing on laboratory animals.

Fritzsche is in error when he writes: "Maharry is simply misinformed for failing to see that the animal rights movement encompasses concern for the environment and a host of other human rights issues."

In my address it was emphasized that for more than 150 years organizations with genuine concern for the welfare of animals have served as the conscience of society. During the past 10 years, however, the animal rights movement has evolved into a vast industry, characterized by an increasing militancy. I documented criminal acts such as: attempted murder, arson, burglary, grand theft, breaking and entering, and terrorist bombings, all committed under the banner of the animal rights movement. These wanton criminal acts are not compatible with environmental concern and a host of human rights issues.

James Maharry, MD
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Corrections

In the article by Helmrich, Stubbs, and Stoerker entitled "Fetal maternal laryngeal papillomatosis in pregnancy: A case report" (AM J OBSTET GYNECOL 1992;166:524-5), on page 524, the title should have been "Fatal maternal laryngeal papillomatosis in pregnancy: A case report."

In the article by Vintzileos et al. entitled "Relationship between biophysical activities and umbilical cord blood gas values" (AM J OBSTET GYNECOL 1991;165:707-13), page 711, Table VI is entitled "Umbilical artery blood gas values associated with compromised biophysical activities"; it should have been "Umbilical vein blood gas values associated with compromised biophysical activities." The correction that appeared in the February 1992 JOURNAL (1992;166:772) erroneously listed Table V as the incorrectly titled table.

In the article by Feinberg et al. entitled "Increased progesterone concentrations are necessary to suppress interleukin-2–activated human mononuclear cell cytotoxicity (AM J OBSTET GYNECOL 1991;165:1872-6), on page 1873, between the paragraphs entitled "Effector cells" and "Cytotoxicity assay" the following paragraph should have appeared:

Target cells. Chang liver cells were grown to confluence in supplemented minimum essential media in 25 cm^2 culture flasks. The cells were removed with trypsin (0.25%) and ethylenediaminetetraacetic acid (0.02%) in saline solution, washed in 10 ml of supplemented minimum essential media, and centrifuged at 200*g* for 10 minutes. The pellet was resuspended in 150 μl supplemented minimum essential media and radiolabeled with 50 μCi sodium chromate (Dupont/New England Nuclear, Cambridge, Mass.). After 90 minutes of incubation at 37° C in 95% air and 5% carbon dioxide, the cells were layered over 3 ml of inactivated fetal calf serum, centrifuged for 10 minutes at 200*g*, washed three times in supplemented minimum essential media, and resuspended to a final concentration of 1×10^5 cells per milliliter in supplemented minimum essential media.

ITEMS

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Office Gynecology, April 20-24, 1992, The Hyatt Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, PA/C, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

The 2nd International Conference on the Extracellular Matrix of the Reproductive Tract will be held May 20-23, 1992, in Toronto, Ontario, Canada. The program includes plenary sessions, mini symposia, free communication, and posters. For further information regarding program content contact: Phyllis C. Leppert, MD, PhD, University of Rochester School of Medicine and Dentistry. Tel.: (716) 336-3784; fax: (716) 338-3798. For registration information contact: Office of Continuing Professional Education, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Box 677, Rochester, NY 14642-8677. Tel.: (716) 275-4392; fax: (716) 473-1482 (attn: Box 677).

Second Congress of the European Society of Contraception, Astir Palace Complex, "Nafsika" Hotel, Vouliagmemi, Athens, Greece, May 6-9, 1992. Secretariat: c/o G. Creatsas, MD, FACS, 9 Kanari St., Athens 10671, Greece. Tel.: +301-3624367, +301-7770850. Fax: +301-3620484, +301-3229448.

Trends in Obstetrics & Gynecology—International Symposium, November 6 to 8, 1992, Taipei, Taiwan. Sponsored by Chang Gung Memorial Hospital, Chang Gung Medical College, and the Association of Obstetrics & Gynecology of the Republic of China. For more information please contact: Secretariat, Trends in Obstetrics & Gynecology—International Symposium, c/o Chang Gung Memorial Hospital, 5 Fu-Hsing St., Kwei Shan, Tao Yuan, Taiwan, Republic of China. Tel.: 886-3-3281200 (ext. 2420); fax: 886-3-3285294.

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March 1992. Approx. 320 pages, 123 ills. (Book Code: 02279)
$24.95 (U.S.); $32.50 (Can.)

Order COMPREHENSIVE GYNECOLOGY, 2nd edition by Herbst and COMPREHENSIVE GYNECOLOGY REVIEW, 2nd edition, by Holzman, as a package and receive a discount!
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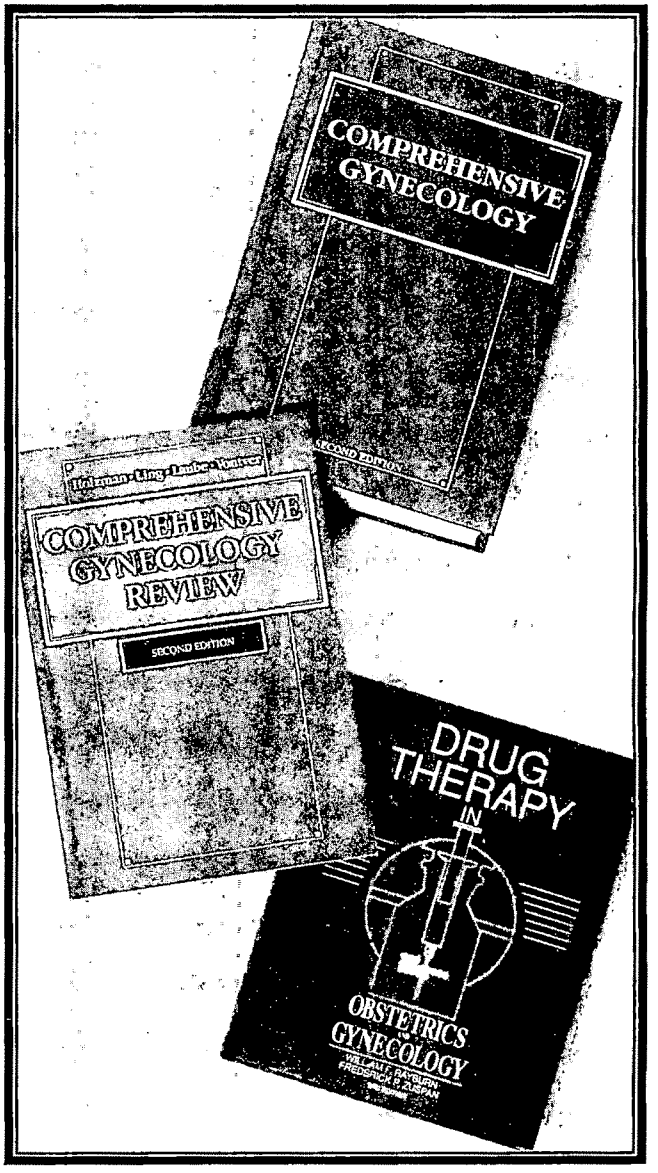
DRUG THERAPY IN OBSTETRICS AND GYNECOLOGY

William F. Rayburn, MD; and Frederick P. Zuspan, MD; with 45 contributors.

Rapid changes in the field of pharmacology make this comprehensive text/reference an invaluable tool for you when dealing with drugs that are primarily or exclusively used to treat women.

- Discusses patient concerns and characteristics, indications, and contraindications associated with each drug.
- Packaged with a **FREE** copy of QUICK REFERENCE TO DRUG THERAPY IN OB-GYN, by Rayburn.

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$49.00 (U.S.); $63.75 (Can.)



New!

A TEXT AND ATLAS OF INTEGRATED COLPOSCOPY

Malcolm C. Anderson, FRCPath, FRCOG; J.A. Jordan, MD, FRCOG; A.R. Morse, CFIAC; and F. Sharp, MD, FRCOG

- Provides "how-to" information in an atlas filled with detailed illustrations.
- Shows how to use the colposcope in the diagnosis, treatment, and management of dysplasia.
- Covers the histological and cytological means of diagnosis, as the complement to colposcopy.

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New!

GYNECOLOGIC & OBSTETRIC SURGERY

David H. Nichols, MD; with contributors.

Written by the masters, this is an authoritative treatise covering all clinical and technical skills necessary to care for gynecologic patients requiring operative management.

- Covers preoperative preparation, operative technique, postoperative care, and management of common problems and complications.
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This is the "gold standard" reference text on diagnosis and treatment of common gynecologic malignancies for OB/GYN residents and practitioners, oncologists, and OB/GYN nurse practitioners.

- New material covers management of the ICU patient and of the elderly or postmenopausal patient.
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- Illustrations have been updated and include new cancer staging diagrams.

October 1992. Approx. 820 pages, 275 ills.
(Book Code: 06756) $85.00 (U.S.); $110.50 (Can.)

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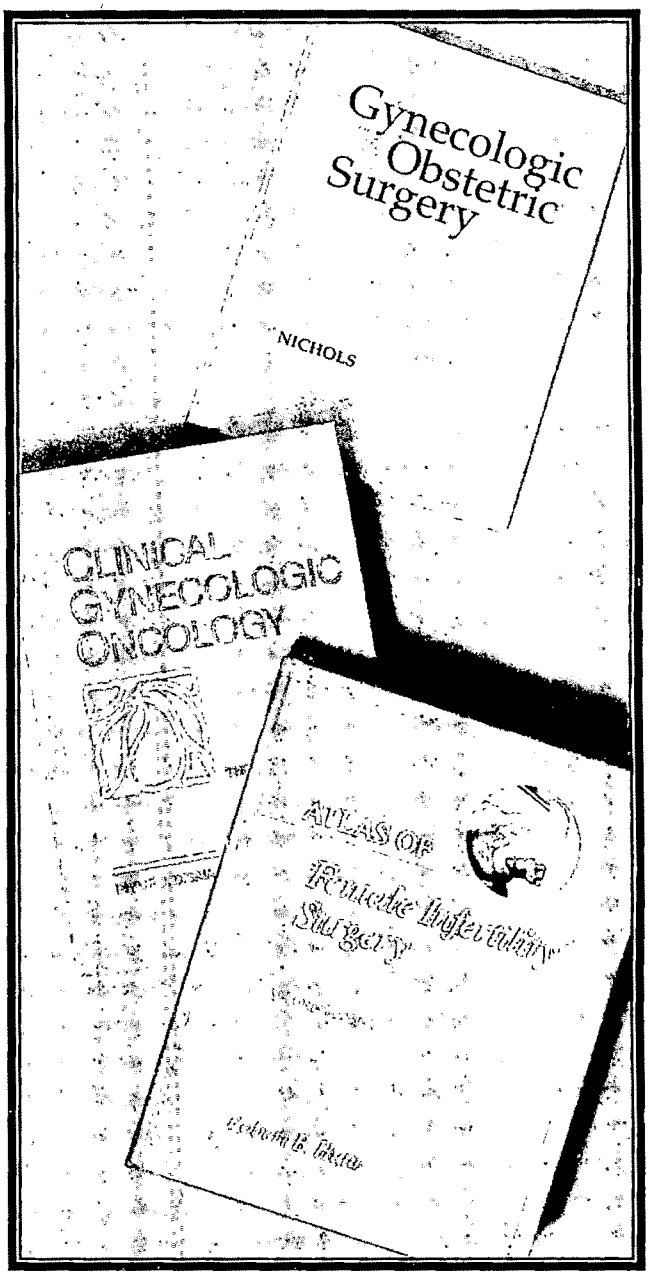
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Robert B. Hunt, MD; with 16 contributors.

"This is an outstanding book. It is the indispensable, invaluable, and definitive work in the field." (New England Journal of Medicine, review of the first edition.)

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1992. 544 pages, 688 ills. with 65 in color. (Book Code: 22450)
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The Department of Obstetrics, Gynecology, and Reproductive Biology at Brigham and Women's Hospital, a major teaching hospital of Harvard Medical School, seeks a full-time Obstetrician/Gynecologist to direct its ambulatory care programs.

Strong interpersonal and management skills, as well as commitment to clinical care, quality improvement, and teaching are essential.

Responsibilities include overseeing the medical management of all ambulatory OB/GYN care in the hospital and its satellite centers.

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Academic rank at Harvard Medical School will be commensurate with experience, training, and achievements. Send inquiry and curriculum vitae to:

Kenneth J. Ryan, M.D.
Chairman, Department of OB/GYN
Brigham and Women's Hospital
75 Francis Street, ASB1-3-073
Boston, MA 02115

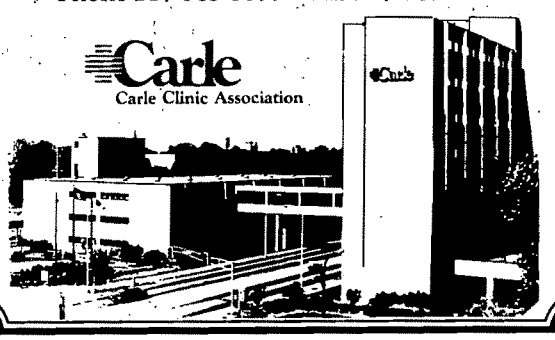
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OB/GYN Physicians needed to join Carle Clinic Association, a 225 physician, multi-specialty group practice in central Illinois with opportunities at the main campus as well as three additional sites - Urbana, Bloomington, Danville and Mattoon/Charleston. These communities offer recreational, cultural and educational amenities. Benefit package includes a competitive salary with partnership and income sharing plan after two years, professional liability insurance, health, life and disability insurance and generous vacation and meeting time. Contact:

Robert C. Parker, Jr., MD
Medical Director
Carle Clinic Association
602 W. University, Urbana, Illinois 61801
Phone 217-383-3399 - Fax 217-383-3163

Carle
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BRONSON

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Kalamazoo, Michigan

A practice opportunity is available for BE/BC Ob/Gyns to join a well-established, busy hospital-based group practice. Excellent salary and malpractice insurance are among the many benefits of this opportunity. Relocation and interviewing expenses will also be provided.

Administrative prerequisites have been established for a Nurse Midwifery Service for this practice and recruitment is also underway for experienced CNMs.

Bronson Hospital, the 442-bed tertiary care facility for southwestern Michigan, has a 500-member medical staff which includes 31 Ob/Gyns, 3 perinatologists and 6 neonatologists. An average of 3,400 deliveries are performed annually at Bronson.

Kalamazoo, located midway between Chicago and Detroit, offers diverse cultural opportunities. Lake Michigan's sandy beaches and dunes are only minutes away and numerous lakes, hills and forests provide boundless recreational activities. Excellent schools, several colleges and a major university further contribute to a high quality life-style. Real estate is affordable and one can be in the beautiful countryside within 10 minutes.

For more information about this practice opportunity, please call or send CV to:

Debra Hartman, Bronson Administrative Services
252 E. Lovell St., Kalamazoo, Michigan 49007
(800) 594-9022 or (616) 341-6331

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FACULTY POSITIONS
GENERALIST OBSTETRICIAN/ GYNECOLOGIST
MATERNAL/FETAL MEDICINE SUBSPECIALIST
GYNECOLOGIC ONCOLOGIST
REPRODUCTIVE ENDOCRINOLOGIST

The James H. Quillen College of Medicine of East Tennessee State University offers an excellent opportunity to physicians of these specialties who are Board Certified or Board Eligible. This opportunity offers both tangible and intangible benefits sometimes not found in larger cities. Our emphasis is on excellence in service to patients, education, and research. These Faculty positions, both Tenure track and Grant-supported, are at the Assistant/Associate Professor level and offer very competitive salaries and excellent fringe benefits. The Gynecologic Oncologist position is funded by an outside source. The Tri-Cities area boasts of good schools and suburban living in beautiful Northeast Tennessee with easy access to various cultural activities. Our outdoor sports include all water sports, snow skiing, the Appalachian Trail, and numerous scenic attractions. Our medical referral base consists of 1.3 million people within a 50 miles radius. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman,
Dept. of OB/GYN
James H. Quillen College
of Medicine
East Tennessee State University
Box 70,569
Johnson City, TN 37614-0569

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Perinatologist Needed in Coastal New Jersey

2 member group is seeking a third perinatologist (BE/BC). We are active practitioners of perinatology and we also serve as integral members of the teaching team for a residency in Obstetrics and Gynecology.

We engage in both in-patient and out-patient activities, and all skills related to maternal fetal medicine may be used within our practice. Strong ultrasound skills are required, and the current practice consists of antenatal diagnosis, genetic counseling, amniocentesis, CVS and PUBS. We will insist on comfort and competence in basic obstetrical practice.

We are located about 1.5 hours from both New York City and Philadelphia, and the area provides a suburban life style.

Contact: Glenn Herman, M.D.
David Wallace, M.D.
Telephone: (908) 870-3600
Fax: (908) 870-0119

MATERNAL/FETAL SUBSPECIALISTS
EASTERN PENNSYLVANIA

Lehigh Valley Hospital, an 830-bed tertiary care teaching hospital, is seeking additional faculty including two full-time maternal/fetal medicine subspecialists: a division chief and an associate. The physicians' roles will include consultative patient care, program development, student and resident education and research if desired. The opportunity exists to customize the role to your particular career goals. Candidates should be board certified or active candidates for certification in maternal/fetal medicine.

Lehigh Valley Hospital is a regional Perinatal Center with over 3,300 births annually, of which 40 percent are high-risk. The department is supported by a 20-bed Level III NICU and four full-time neonatologists. The hospital serves as a clinical campus of Hahnemann University and maintains a free-standing, fully-accredited OB/GYN residency program.

Allentown, Pennsylvania is a cosmopolitan city, 60 miles north of Philadelphia and 80 miles west of New York City. The area has excellent school systems, nine colleges and universities and diverse cultural offerings. An excellent compensation package has been designed to recruit qualified candidates. Please send CV to **Carol Voorhees, Vice President, Lehigh Valley Hospital, 1243 S. Cedar Crest Boulevard, Suite A, Allentown, PA 18103, or call (215) 821-3090.** EOE.

NORTHERN CALIFORNIA– BAY AREA
PERINATOLOGIST

Outstanding opportunity for BE/BC perinatologist to join solo private practice. The Good Samaritan Hospital is a private, level III center with over 4,000 deliveries annually. NICU is staffed by five neonatologists. Excellent institutional support with full range of prenatal diagnostic services and state-of-the-art Antepartum Testing unit. Experience desired in CVS, prenatal diagnostic ultrasound, PUBS and complicated pregnancies.

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Call (408) 559-2258 or send CV to:

Kevin J. Gomez, M.D.
2425 Samaritan Drive
San Jose, CA 95124



Women's Health Care of Hinsdale, a four-person general obstetrics and gynecology group, is looking for an energetic, BC/BE physician to join them full-time in private practice. Our physicians are exclusively on staff at Hinsdale Hospital, a 459-bed facility with 10 LDR's, a Level II+ nursery, 24-hour neonatology services, and 24-hour in-house anesthesia services dedicated to Labor and Delivery.

Hinsdale, Illinois is located 17 miles southwest of downtown Chicago in a beautiful, upscale western suburb. Our location offers us the opportunity to take advantage of the best that Hinsdale has to offer – exceptional schools, housing, shopping, dining – in addition to being able to enjoy things that are uniquely Chicago – The Art Institute, the Chicago Symphony Orchestra, world-class professional sporting events...all just minutes away.

Our salary and benefits package is highly competitive. Since we would like to discuss our opportunity with you in more detail, please reply with your CV to Margaret Pfister, M.D., Women's Health Care of Hinsdale, 333 Chestnut, Suite 201, Hinsdale, IL 60521 or call 708/920-2229.

PERINATOLOGIST OGDEN, UTAH

An outstanding opportunity exists for a perinatologist to set up a consultative practice in Ogden, Utah. The physician will be an employee of McKay-Dee Hospital, a 415-bed community facility. Office space, personnel and equipment will be provided by the hospital. The successful candidate will be an integral part of Intermountain Health Care's Perinatal Group.

Ogden, Utah is a beautiful community of 69,000, with a service population of 260,000. There is an abundance of outdoor recreation available, including skiing, snowmobiling, hunting, fishing, boating, swimming and camping. A variety of cultural events entertain and amuse Ogden residents, such as the symphony, the ballet, museums, art galleries and festivals. Ogden is a short 40 minutes from Salt Lake City where more professional sports and cultural events may be found.

For more information, send a CV to:
IHC Physician Placement Services
P.O. Box 57010
Murray, UT 84157-0010
or call toll-free, 1-800-888-3134

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James Schwering
P.O. Box 1648
Eugene, OR 97440
503-686-3850

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St. Agnes Hospital in the City of Baltimore solicits your interest in the position of Chairperson, Obstetrics & Gynecology

The medical staff and administration seek an individual around whom a strong clinical department and residency program can be built.

St. Agnes has the caseload (2700 deliveries/year), a Level III NICU (22 bassinets), a stable staff of nurses, new midwivery service, and new obstetrical/neonatal unit (twelve LDR's/90 bassinets) scheduled to open next year.

There are established relationships with Johns Hopkins University and the University of Maryland; most staff have faculty appointments. St. Agnes has approved residencies in Internal Medicine and General Surgery and a Pediatric program affiliated with Hopkins. A new residency in OB/GYN is a priority for 1993.

For details, call CAROLE GREGORY at 1+800/220-6331 or send your CV to MEDQUEST, P.O. Box MQ, Devault, PA 19432.

MedQuest INC.

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Director of Maternal Fetal Medicine
Director of Gynecologic Oncology
Director of Reproductive Endocrinology
Generalist in Obstetrics-Gynecology

The Department of Obstetrics-Gynecology at the Medical College of Ohio is actively recruiting outstanding professionals to fill important clinical positions as we are embarking on the rebuilding of the academic and patient service program.

The Medical College of Ohio has a modern, new ambulatory services facility, is involved in the community's only Level 3 perinatal center, and boasts a spectacular academic campus.

Qualifications include board eligibility or certification and a commitment to teaching. Salary and academic rank are commensurate in candidate's training and experience.

Send inquiries and curricula vitae to Louis Weinstein, M.D., Professor and Chairman, Department of Obstetrics-Gynecology, Medical College of Ohio, P.O. Box 10008, Toledo, Ohio, 43699.

The Medical College of Ohio is an equal opportunity/affirmative action employer.

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Excellent opportunity for BE/BC OB-GYN to join rapidly expanding private practice in desirable northern NJ area with quick access to NYC. Modern well-rounded office including colposcopy-LEEP, cryosurgery and ultrasound. Highly competitive salary and generous benefits package leading to full partnership. Position available immediately or July 92. Mail CV to: Richard E. Marki, MD, 744 Broadway, Bayonne, NJ 07002 or Fax (201) 858-0467. All replies held in confidence.

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North Memorial Perinatal Center is seeking a board eligible/board certified Maternal Fetal Specialist to join Harry Farb, M.D. in the practice of Maternal Fetal Medicine. This position is best suited for an individual who likes personal, direct, hands-on patient care, teaching and outreach activities. Experience in CVS and PUBS is required. An excellent financial package, support services and personal and professional growth is offered by this opportunity.

Send CV to:

Harry Farb, M.D.
Medical Director
North Memorial Perinatal Center
3300 Oakdale North
Robbinsdale, Minnesota 55422
(612) 520-5950

OBSTETRICIAN-GYNECOLOGISTS, BC/BE needed to join well established solo physician, interested in building a family centered group practice. Position available in San Antonio, Texas, area rich in both cultural and recreational opportunities, and near medical school.

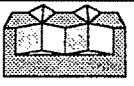
Please direct inquiries to:

Manager
P.O. Box 291229
San Antonio, Texas 78229

OB/GYN
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Enjoy sharing the challenge of an Obstetrics and Gynecology practice with two Perinatologists, Certified Nurse Midwives and a Certified Nurse Practitioner, while living where most people vacation. We desire a Board Certified or Board Eligible OB/GYN surgeon. A modest call schedule and comfortable benefits are a part of our offer. We have a positive, patient oriented staff. Northern Arizona is a great place to raise a family. Please send letter of interest and Curriculum Vitae to:

Perinatal Associates
of Northern Arizona
ATTN: Ritamarie Lesauski
77 West Forest, Suite 205
Flagstaff, AZ 86001
or call (602) 773-2552



The Mount Sinai Medical Center of New York

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Excellent opportunities exist for Board-certified or eligible physicians to join our internationally recognized faculty consisting of twenty full-time clinicians, five research investigators and sixty-five voluntary attendings, as well as twenty-eight residents and fellows in each of three subspecialty divisions.

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These individuals will have primary teaching and clinical responsibilities on the obstetrical and gynecologic services. The selected individuals will be appointed to the departmental faculty and have the opportunity to pursue private practice opportunities.

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The Division of Maternal Fetal Medicine is a major referral center for high risk pregnancies and fetuses at risk or suspected to have anomalies, and offers a broad range of services including vaginal and abdominal ultrasound, fetal blood samples, fetal echocardiography and a large spectrum of intrauterine procedures. Faculty members have service and consultative responsibilities along with those of teaching medical students, residents and maternal fetal medicine fellows. We seek individuals who are interested in careers in academic medicine, research, teaching and patient care.

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For consideration, please direct your letter, curriculum vitae and the names of three references to: Richard L. Berkowitz, Professor and Chairman, Department of Obstetrics, Gynecology and Reproductive Science, Box 1170, The Mount Sinai Medical Center of New York, One Gustave L. Levy Place, New York, NY 10029-6574. An Equal Opportunity Employer.

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Send CV To: Joyce Leslie, M.D.,
Planned Parenthood of Tompkins County,
314 West State Street, Ithaca, NY 14850

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Seeking **recently-certified** MD to associate with established surgeon in an international referral practice. Practice limited to gynecology with emphasis in advanced laparoscopy and the non-medical treatment of endometriosis. Located in the Pacific Northwest, minutes away from year-round outdoor recreation. Should have **above average** basic surgical skills. Familiarity with Macintosh computers, dBASE, video production helpful. Clinical research, publication, speaking and travel are possibilities. Send CV, recent photo, 3 references in support of your surgical expertise and why you think you are the person for this position to:

John Wood
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PO Box 19949
Houston, TX 77224

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is seeking applicants for two (2) full-time faculty positions for a new women's health center and academic program at its Milwaukee Clinical Campus.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department supports a residency program, the largest perinatal service in Wisconsin (5500 deliveries), consultative ultrasound, genetic and infectious disease programs, and reproductive/endocrine services (including an IVF program). The department is affiliated with Planned Parenthood of Wisconsin and St. Luke's Medical Center in Milwaukee.

Qualifications include a board candidacy or certification in obstetrics and gynecology and a commitment to teaching residents, medical students, and clinical research. Qualifications for the two positions for GENERAL OB/GYN can take into consideration special individual interests. Special expertise of interest for the department are:

—STATEWIDE FAMILY PLANNING
(Part-time medical director, Planned Parenthood)
—OPERATIVE LAPAROSCOPY, HYSTEROSCOPY
—UROGYNECOLOGY
—OB/GYN ULTRASOUND

Private practice opportunities are also available in an associated offsite multispecialty clinic and all faculty members participate in a group practice at the institution. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics and Gynecology
University of Wisconsin Medical Schl.
600 Highland Avenue, H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
University of Wisconsin Medical Schl.
Sinai Samaritan Medical Center
2000 West Kilbourn Avenue
Milwaukee, WI 53233
(414) 937-5600

The University of Wisconsin Medical School is an equal opportunity employer

OB/GYN Subspecialists

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Perinatology
Reproductive Endocrinology
Urogynecology

The University of South Dakota School of Medicine's Department of Obstetrics and Gynecology is building a full time academic team. We are located in Sioux Falls, South Dakota, a regional medical community of 120,000 serving South Dakota and three neighboring states.

Challenging positions are available for qualified candidates interested in pioneering medical education, research development and clinical practice.

A new comprehensive University-Health Science Center is being constructed which will house our program. Outreach and Indian Health opportunities are also available.

Financial package and incentives are competitive and commensurate with experience, credentials and rank.

Please send Curriculum Vitae to: R. H. Wiebe, M.D. Professor and Chairman, Department of Obstetrics and Gynecology, c/o Physician Placement Program, 1100 South Euclid Avenue, Sioux Falls, South Dakota 57117-5039 or call 800-468-3333 for more information.

CHAIRMAN DEPARTMENT OF OBSTETRICS/GYNECOLOGY

Saint Joseph Hospital and Health Care Center, a 497 bed hospital, affiliated with Northwestern Medical School, seeks a full time Chairman for its Department of OB/Gyne. The Senior attending, Board Certified candidate will direct the department and residency program in Obstetrics and Gynecology, expand its services and assure quality patient care. Demonstrated accomplishments in medical education and administration are essential.

Submit a C.V. to:

P.C. Williams, M.D., Chairman
Search Committee
Saint Joseph Hospital and Health Care Center
2900 North Lake Shore Drive
Chicago, IL. 60657

DIRECTOR MATERNAL FETAL MEDICINE

Department of Obstetrics and Gynecology
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School

The Robert Wood Johnson Medical School invites applications for **Director of Maternal-Fetal Medicine.** The position of Director requires an individual with an established and broad understanding of medical education, research, patient care as well as effective administrative skills. Applicants should possess qualifications commensurate with those of an Associate Professor/Professor of Obstetrics and Gynecology as defined by the Robert Wood Johnson Medical School.

The Division is located at St. Peter's Medical Center, the largest obstetrical and neonatal program in the State. Maternal-Fetal Medicine consists of five perinatologists, two MFM Fellows, four sonographers, a geneticist, two research nurses and one epidemiologist/biostatistician. The Robert Wood Johnson Program supports 7,000 deliveries per year.

Interested individuals should forward their curriculum vitae to:

Robert A. Knuppel, M.D., M.P.H.
Professor and Chairman
UMDNJ-Robert Wood Johnson Medical School
One Robert Wood Johnson Place-CN 19
New Brunswick, New Jersey 08903-0019

The UMDNJ is an Affirmative Action/Equal Opportunity Employer M/F/H/V and a member of the University Health System of New Jersey

One full-time position is available in the Department of Obstetrics and Gynecology, University of California, Davis, Medical Center, Sacramento, as Director of the Division of Maternal/Fetal Medicine with concurrent appointments to the faculty of the University of California, Davis, School of Medicine. Applicants for this perinatologist position should be Board eligible or certified in Obstetrics and Gynecology and must have successfully completed subspecialty training in Maternal/Fetal Medicine with subsequent Specialty Board eligibility and/or certification in Maternal Fetal Medicine. Applicants must possess or be eligible for medical licensure in the State of California. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities or potential for such activities. Applicants must demonstrate experience in prenatal diagnosis, ultrasound and genetics.

Responsibilities of this position include direction and responsibility for research activities in the division, both inpatient and outpatient case management, surgery and procedures specific to high risk obstetrics, participation in teaching activities with residents, interns and medical students, development and/or participation in research activities and involvement in University and Hospital committees. Serves as Administrative Director of the Division.

Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
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Department of Obstetrics/Gynecology
University of Wisconsin Medical School
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Madison, WI 53792

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Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
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Registration: Before 6/30/92: $150; After 6/30/92: $175. Checks payable to: The Perinatal Research Institute (in U.S. dollars)

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For abstract form & information contact: Dr. Reginald Tsang or Ms. Renee McKenzie, The Perinatal Research Institute, Univ of Cincinnati, 231 Bethesda Ave, Cincinnati, OH 45267-0541; (513) 558-1771

One full-time position is available in the Department of Obstetrics and Gynecology, University of California, Davis, Medical Center, Sacramento, as a perinatologist on the Maternal/Fetal Medicine Service with concurrent appointments to the faculty of the University of California, Davis, School of Medicine. Applicants should be Board eligible or certified in Obstetrics and Gynecology and must have successfully completed subspecialty training in Maternal/Fetal Medicine with subsequent Specialty Board eligibility and/or certification in Maternal Fetal Medicine. Applicants must possess or be eligible for medical licensure in the State of California. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities or potential for such activities. Applicants must demonstrate experience in prenatal diagnosis, ultrasound and genetics.

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Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiform, Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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